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Official Organ of the Society for the Study of Development and Growth

GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

PERIODICAL ROOM
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VOLUME V, NUMBER 4
PAPERS 180-189

DECEMBER, 1941 **$5.00 A YEAR**

GROWTH

A journal for the coördination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a coöperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy, and anthropology; just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines, and hormones.

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Application for entry as second class matter at the post-office at Worcester, Mass., is pending.

Growth, 1941, **5**, 329-350.

THE EFFECTS OF l-PROLINE, l-HYDROXYPROLINE, AND l-ASPARTIC ACID ON DIFFERENTIATION OF RABBIT OVA: WITH A NOTE ON THEIR CLINICAL USE IN ANEMIA*

BERNARD J. MILLER, VINCENT W. CIACCI, AND STANLEY P. REIMANN

The Lankenau Hospital Research Institute, Philadelphia, Pennsylvania
(Received for publication, August 1, 1941)

Ova are complete organisms. Therefore, they have many theoretical and practical advantages over ordinary tissue culture material in studies of cell behavior under the influence of various physical and chemical factors. Thus the development of methods for culture of mammalian ova, both fertilized and unfertilized, is another powerful tool for the study of cells.

The present series of studies are concerned with the effect of various amino acids and some nuclear compounds on developmental processes in rabbit ova.

From Hammett's study on hydra (Obelia Geniculata) among other conclusions, the following is unquestioned in these animals. Each one of the amino acids has its own particular part to play in some phase or other of growth and development. These studies covering a period of 10 years, have included all of the known amino acids with the exception of betahydroxy-glutamic acid and several nuclear compounds (details and lit. see 1).

It is our intention to extend to rabbit ova studies of the effects of these amino acids from Obelia Geniculata and several other organisms which Hammett and his co-workers have done. Previous reports are of cystine and methionine (2); the present report deals with the effects of l-proline, l-hydroxyproline, l-aspartic acid, combinations of the latter two and liver extract as a control.

APPARATUS AND METHOD

Ova were recovered from the Fallopian tubes of rabbits about 48 hours after copulation by the usual flushing out technic. They were then cultured either in the circulating apparatus or Carrel flasks.

*Aided by a grant from Mrs. L. Elizabeth Nax, Philadelphia, Pennsylvania.

Pooled serum from several does or from the doe from which the ova were recovered, was used as a medium in these experiments. Blood was obtained by heart puncture. The syringe was first rinsed with cold sterile Tyrode's solution to minimize danger of hemolysis, the slightest amount of which is inhibitory. The clot was then floated from the wall of the tube and the mixture stored in the refrigerator over night. Serum was obtained the next day by centrifugation.

The animals were anesthetized with ether and the uteri and Fallopian tubes excised. It is well to dissect the mesosalpinx from the tubes to prevent buckling during flushing. About 2 cc. of serum or a mixture of Tyrode's solution and serum are sufficient to wash out all ova. The ova are recovered by means of sterile pipettes and transferred to small or large Carrel flasks. The quantities of serum varied between 2 cc. and 5 cc.

Compounds to be studied were dissolved in sufficient Tyrode's solution to make a M/100 solution which was then filtered through an L.5 Chamberlain filter out of which proportions of the sterile filtrate were prepared in appropriate amounts of clear serum to obtain proper dilutions.

Ova ready to be studied histologically were removed from the hanging drop, washed in Tyrode's solution and dropped into Allen's modification of Bouin's fluid. They were dehydrated by the drop method with two changes of 95 per cent and absolute alcohol preceding clearing with cedar oil. Two changes of cedar oil are advantageous and they are permitted to remain in the last change of cedar oil for 24 hours. Three changes of paraffin were used. Eggs were kept under direct observation during dehydration and infiltration. This is done by using small embryological dishes placed on a hot plate during infiltration and observed with a long arm binocular microscope.

The ova were oriented alongside a piece of embryonic liver or spleen and then rapidly embedded. Sections were cut at 5 micra in such a manner that the knife struck the marker first. They were then stained with Delafield's hematoxylin or Mayer's hemalum followed by one per cent alcoholic eosin.

Varying quantities of l-proline, l-hydroxyproline, l-aspartic acid and combinations of l-hydroxyproline and l-aspartic acid in equi-molar concentration, were placed in small amounts of serum rather than large amounts, for ova cultured in large quantities of serum seem to exhibit more adaptive powers to alter their metabolic equilibrium. Concentrations used were M/10,000, M/100,000, and M/200,000.

The number of ova recovered from individual rabbits varied from 5 to 10. They were divided into control and experimental ones, the only difference being the addition of the compounds to their media.

In the description below, typical findings in all experimental ova are given with what exceptions were noted, all in relation to their respective controls.

Results

The following experiments were performed with M/10,000 l-proline.

Three fertilized ova obtained approximately 48 hours after copulation from doe *RA*3 were cultured for three days. Compared to two controls from the same doe they showed: (*a*) smaller cells with more definitely defined cell walls; (*b*) 12 fragments of the main cell mass were clearly visible in the perivitelline space and varied between 15 and 40 micra in diameter. When sectioned and stained, their cellular nature and small size were definite. Nothing similar was present in the control ova which had segmented regularly. Their main cell mass was slightly larger and showed no herniation or fragmentation.

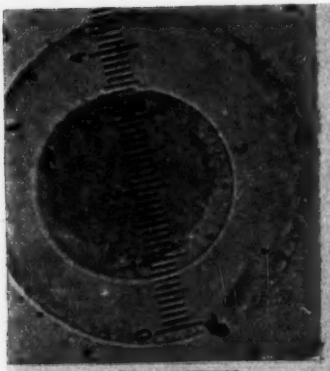
The following experiments were performed with M/100,000 and M/200,000 l-proline. Two ova were used for each concentration and comparisons were made with two controls all from doe *RA*4. The results in this case were similar to those observed with M/10,000 l-proline with the exceptions: (*a*) the cell fragments were smaller; (*b*) tissue fragments have migrated from the main cell mass to the perivitelline space (Figure 1, B).

With M/200,000 the effects were the same except that no fragments were found but only single cells (Figure 1, C).

The controls in this case were similar to the previous ones, i.e., the main cell cluster showed a uniform periphery and no fragments. Cells of the main mass were larger and had less definite cell walls (Figure 1, A). Repeat experiments substantiated the above findings in six ova from doe *RA*12 (Figure 1, D).

The results of three ova from doe *RA*13 cultured in a 35 ml. circulating culture of M/10,000 l-proline for five days are as follows:

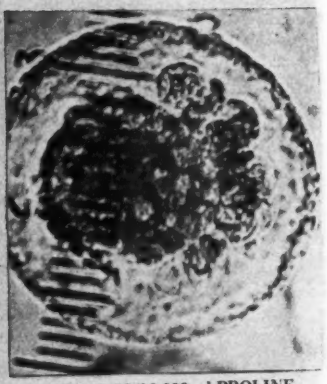
Small accumulations of differentiated cells were seen throughout the cellular mass (Figure 1, E and F). These cells stained heavily with hematoxylin, showed little cytoplasm, and were about 7-9 micra in diameter. They might be erythroblasts, at least they were non-granular and were differentiated from the others.



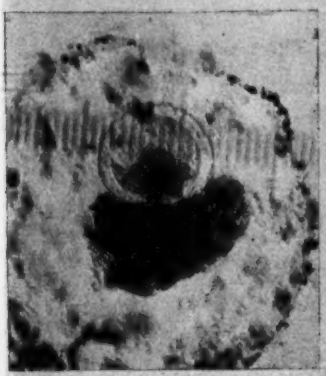
A, RA4 CONTROL

B, RA4 M/100,000—l-PROLINE

C, RA4 M/200,000—l-PROLINE



D, RA12 M/10,000—l-PROLINE



E, RA13 M/10,000—l-PROLINE

F, SECTION—RA13

FIG. 1

FIGURE 1

A. Control Ovum *RA*4. Cultured 72 hrs.

B. Experimental Ovum *RA*4. Cultured 72 hrs. in M/100,000 l-Proline showing perivitelline tissue fragments.

C. Experimental Ovum *RA*4. Cultured 72 hrs. in M/200,000 l-Proline showing single cells in perivitelline space.

D. Experimental Ovum *RA*12. Cultured 72 hrs. in M/10,000 l-Proline showing perivitelline tissue fragments.

E. Experimental Ovum *RA*15. Cultured 120 hrs. in 35 ml. circulating culture of serum made M/10,000 with l-Proline.

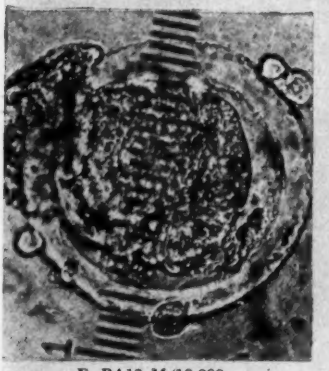
F. Section of Ovum *RA*13 showing small highly differentiated cells.

A, RA14 M/10,000—l-PROLINE

B, SECTION—RA14

C, RA13 CONTROL

D, SECTION—RA13



E, RA13 M/10,000—
l-HYDROXYPROLINE

F, SECTION—RA13

FIG. 2

FIGURE 2

A. Experimental Ovum *RA*14. Cultured 120 hrs. in 5 ml. of serum made M/10,000 with l-Proline.

B. Section of Ovum *RA*14.

C. Control Ovum *RA*13. Cultured 72 hrs. in 5 ml. of serum.

D. Section of Control Ovum *RA*13. Showing characteristic uniformity of trophoblast in all control ova.
B. Trophoblast
C. Blastocyst Cavity
D. Inner Cell Mass

E. Experimental Ovum *RA*13. Cultured 72 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.

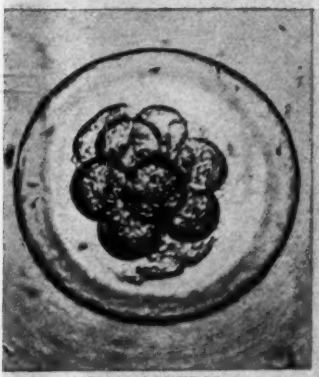
F. Section of Experimental Ovum *RA*13. Cultured 72 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.
A. Hematocytoblast-like Cell
B. Trophoblast
C. Blastocyst Cavity

A, HEMATOCYTOBLAST—LIKE CELL

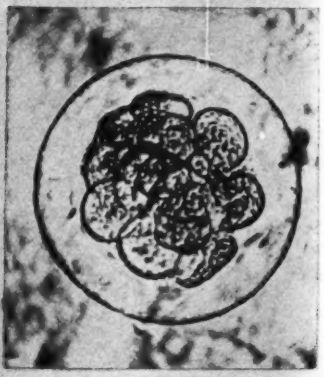
B, RA13 M/10,000—
l-HYDROXYPROLINE

C, SECTION—RA13

D, HEMATOCYTOBLAST—LIKE CELI



E, RA15 CONTROL



F, RA15 CONTROL

FIG. 3

FIGURE 3

A. High Magnification of Hematocytoblast seen in Figure 2, F.
  A. Hematocytoblast-like Cell
  B. Trophoblast
  C. Blastocyst Cavity

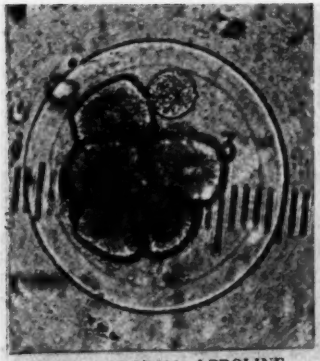
B. Experimental Ovum *RA*13. Cultured 72 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.

C. Section of Experimental Ovum *RA*13. Cultured 72 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.
  B. Trophoblast
  C. Blastocyst Cavity

D. Division of Trophoblast Cell into Hematopoietic Anlage.
  A. Hematocytoblast-like Cell
  B. Trophoblast
  C. Blastocyst Cavity

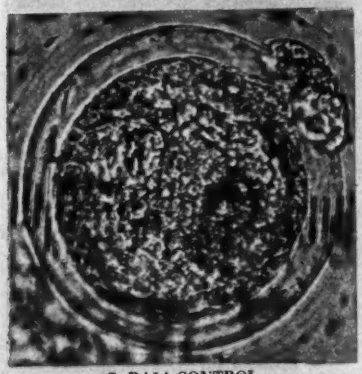
E. Control Ovum *RA*15. Cultured in 5 ml. of serum for 8 hrs.

F. Control Ovum *RA*15. Removed after 24 hrs. of culture.

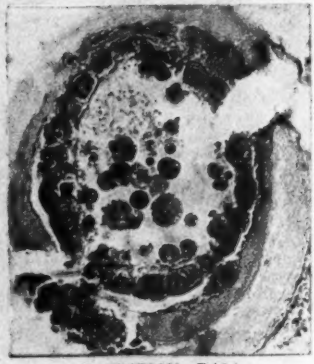


A, RA15 M/10,000—l-PROLINE

B, SECTION—RA15



C, RA14 CONTROL



D, SECTION—RA14

E, RA14 M/10,000—
l-HYDROXYPROLINE

F, SECTION—RA14

FIG. 4

FIGURE 4

A. Experimental Ovum *RA*15. Cultured for 8 hrs. in 5 ml. of serum made M/10,000 with l-Proline.

B. Section of Experimental Ovum *RA*15.

C. Control Ovum *RA*14. Cultured 120 hrs. in 5 ml. of clear serum.

D. Section of Control Ovum *RA*14.

E. Experimental Ovum *RA*14. Cultured 72 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.

F. Section of Experimental Ovum from same group showing:
A. Early Differentiated Cluster
B. Highly Specialized Cell

The following experiments were performed with four ova from doe *RA*14 in 5 ml. serum containing M/10,000 l-proline.

(*a*) After three days, the cells of the main mass were smaller and more clearly differentiated. A small cluster of cells herniated through the zona pellucida into the layer of albumen. Other small masses were within the ovum and apparently related to the trophoblast.

(*b*) After five days, the cluster of cells which had herniated was four times the size of the herniated mass in the controls. There was an exceptionally large number of very small, prematurely differentiated cells within the zona pellucida (Figure 2, A). Five globular masses of cells were visible in the hernia. On section these proved to be anatomically identical with the cells previously postulated as erythroblasts (Figure 2, B).

(*c*) Control ova show no early differentiation and in all respects are similar to normally cleaving ova for this period of development (Figure 5, B).

The final variant of time was in six ova isolated in approximately the nine-cell stage from doe *RA*15 and cultured in M/10,000 l-proline for eight hours. The findings were:

Blastomeres had undergone peripheral flattening and a small spherical mass of cells had been cast out into the perivitelline space (Figure 4, A). On section the cellular nature of the spherical mass was confirmed (Figure 4, B). The cells in the control remained spherical and no differentiated masses were seen (Figure 3, E).

Studies with l-Hydroxyproline

Two 48-hour ova, from doe *RA*13, were cultured for three days. Compared to control ova of the same group cultured for the same period they showed:

(*a*) Fragmented masses in the perivitelline space as was the case with l-proline (Figures 2, E, and 3, B). These varied between 10 to 30 micra in diameter and seemed to average six in number.

(*b*) In addition, an average of five cellular masses migrated through the point of hernia and were seen in the surrounding albumin (Figures 2, E, and 3, B).

The globular masses seen in the perivitelline space and also in the albumen coat were of definite cellular composition (Figure 3, C). The cells composing these masses were small and contained a highly chromatic nucleus. In addition to this, cells of a specialized appear-

ance were seen in the blastocyst cavity and also among the trophoblast cells from which they had apparently been formed. (Figures 2, F, and 3, D). These measured about 15 micra and resembled those seen in sections of ova cultured in l-proline with the large circulating cultures. Large cells which showed considerable chromatin material were seen closely associated with the internal surface of the trophoblast (Figures 2, F, and 3, A). In all respects these cells were of the nature of hematocytoblasts. The nucleus was comparatively large and highly basophilic. Polychromatic cytoplasmic granules were seen. Occasionally one of these cells appeared to be budded off from a trophoblast cell (Figure 3, D).

Sections of control ova show no evidence of premature differentiation (Figure 2, D). No specialized cell, other than cells of the trophoblast and inner-cell mass, were seen in any of the sections (Figure 2, D). The cells of the trophoblast were cuboidal, very uniform, and showed no activity other than that of mitotic activity. No irregularity of cytoplasm was seen. In all respects sections seemed typical for ova in this stage of development.

Additional ova from doe *RA*14 were cultured in 5 ml. of serum made M/10,000 with l-hydroxyproline. Those cultured for three days showed the following:

(*a*) These were slightly smaller than their control (Figure 4, C and E).

(*b*) In hanging drop these ova showed typically more differentiated cells composing the main cell mass. In addition to this, one ovum showed a well defined mass within the ovum proper. Sections of these ova showed the same picture as seen in ova previously studied with l-hydroxyproline (Figures 4, F, and 5, A) while sections of control ova were histologically the same as other controls (Figure 4, D). Those cultured for five days showed the following:

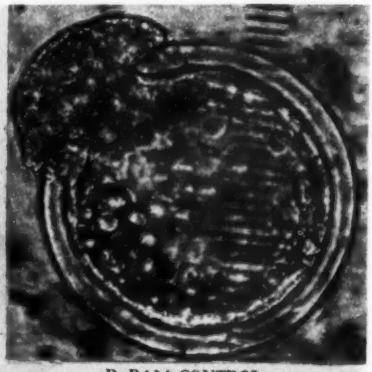
(*a*) There were fewer large cells than in the control ova. The relative proportions of small to large cells was even greater than with experimental ova cultured in l-proline (Figure 5, C).

(*b*) Sections of these ova confirm the above observations. In addition the large mitotically active cells had been replaced by the smaller more highly differentiated cells (Figure 5, C).

Studies with l-Aspartic Acid

Twenty-one fertilized ova obtained 48 hours after copulation from

A, SECTION—RA14



B, RA14 CONTROL

C, RA14 M/10,000—

D, SECTION—RA14

E, RC6 M/10,000—l-ASPARTIC ACID

FIG. 5

F, RC6 M/10,000—l-ASPARTIC ACID

FIGURE 5

A. Section of Experimental Ovum from same group as Figure 4, F.

B. Control Ovum *RA*14. Cultured 120 hrs. in 5 ml. of clear serum.

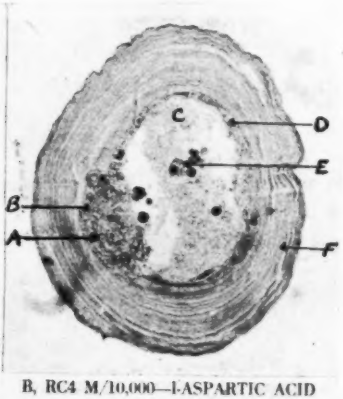
C. Experimental Ovum *RA*14. Cultured 120 hrs. in 5 ml. of serum made M/10,000 with l-Hydroxyproline.

D. Section of Experimental Ovum *RA*14 showing relative increase in number of small cells compared to the large type cells.

E. Experimental Ovum *RC*6. Cultured 72 hrs. 6 ml. of serum made M/10,000 with l-Aspartic Acid.

F. Section of Experimental Ovum *RC*6 showing:
A. Typical Cell

A, RC6 CONTROL



B, RC4 M/10,000—l-ASPARTIC ACID

C, RC4 CONTROL

D, RC10 M/10,000—l-ASPARTIC ACID AND M/10,000—l-HYDROXYPROLINE

E, RC8 LIVER EXTRACT

FIG. 6

F, RC10 M/10,000—l-ASPARTIC ACID AND M/10,000—l-HYDROXYPROLINE

FIGURE 6

A. Section of Control Ovum *RC*6. Note absence of specialized cells.
  A. Blastocyst Cavity
  B. Amniotic Cavity

B. Section of Experimental Ovum *RC*4. Cultured 72 hrs. in 6 ml. of serum made M/10,000 with l-Aspartic Acid.
  A. Beginning Amniotic Cavity
  B. Inner-Cell Mass.
  C. Blastocyst Cavity
  D. Trophoblast
  E. Characteristic Cell produced with l-Aspartic Acid
  F. Albumin Coat

C. Section of Control Ovum *RC*4. Cultured 72 hrs. in 6 ml. of serum. Note high degree of cell uniformity.
  A. Zona Pellucida
  B. Inner-Cell Mass
  C. Blastocyst Cavity
  D. Trophoblast

D. Section of Experimental Ovum *RC*10. Cultured 72 hrs. in an equimolar concentration of l-hydroxyproline and l-aspartic acid. Note typical cell in blastocyst cavity.

E. Section of Experimental Ovum *RC*8. Cultured 72 hrs. in 6 ml. of serum to which had been added a non-toxic amount of Standard Liver Solution.

F. Section of Experimental Ovum *RC*10. Cultured 72 hrs. in an equimolar concentration of l-hydroxyproline and l-aspartic acid.

does *RB*1, *RB*2, *RC*1 to *RC*9 inclusive were cultured in 5 ml. clear serum made M/10,000 with l-aspartic acid for three to five days.

Compared to controls which were studied with the ova of each doe, the following results were uniformly obtained in each experiment:

(*a*) Large typical mononuclear and multinuclear cells measuring approximately 18 micra in diameter were constantly seen in the blastocyst cavity and occasionally in the amniotic cavity closely associated with a thickened trophoblast and embryonic plate (Figure 5, E and F). These were never observed with control ova (Figure 6, A). Small highly chromatic and granular cells were seen in greatest numbers in the angle between the embryonic disc and trophoblast.

(*b*) The nuclei of these large cells appeared resting, peripherally placed and densely granular (Figures 5, E, and 6, B). The limiting cell wall is very apparent. Small round cells may be seen, at times, in close relation to the large round cells.

Twenty-one ova cultured for three days in M/100,000 l-aspartic acid also showed precocious development.

(*a*) In ideal cases only a few large round cells were seen.

(*b*) The blastocyst cavities of the controls were usually smaller than were those of experimental ova. Controls consisted of large fibroblast-like cells with elongate processes (Figure 6, A).

The following experiments were performed with an equimolar concentration of l-hydroxyproline and l-aspartic acid.

Four 48-hour ova obtained from doe *RC*9 and three from *RC*10 were cultured in 5 ml. clear serum. With respect to their controls they showed:

(*a*) Experimental ova showed a large blastocyst cavity.

(*b*) The trophoblasts of experimental ova were much more irregular and more mitotic than were those of control ova for the same group.

(*c*) Many cells of the large round variety were seen. These were everywhere in relation to the trophoblast. The nuclei in these cases were dense and occasionally multilobular (Figure 6, D) as was the case with ova of doe *RA*13 cultured in l-hydroxy-proline. The cytoplasm was densely granular.

With ova cultured in non-toxic[1] dilution of standard liver extract

[1]Each cc. of purified liver solution yields 17.3 mg. solid. Dilutions were prepared by assuming the liver solution to contain 10 to 14 per cent of l-hydroxy-proline and 20 per cent of aspartic acid as based on the analysis of Dakin and West (10).

results were obtained which were indistinguishable from those obtained when ova were cultured in equimolar concentration of both amino acids (Figure 6, E).

Discussion

Ova in the nine-cell stage are not as yet differentiating (Figure 3, E). They are more concerned with increasing their protoplasmic mass by fairly rapid cellular proliferation prior to implantation. As is pointed out in this paper and also by Gregory (3) differentiation is first observed when the peripheral blastomeres of the morula begin to show evidence of flattening (Figure 3, F). This occurs in blastomeres which are apparently the "anlage" of the trophoblast and indicates a potentiality for specific differentiation, resident probably, in specific blastomeres, and controlled by the chemistry of the individual cells. If this is the case one should be able to change the normal sequence of differentiation of multipotent cells by altering the chemical equilibrium or else by making available in excess the compounds necessary for differentiation. This latter method has been used with, of course, unknown effects on actual internal cell chemistry.

Such development of blastomeres may be induced precociously by adding proper amounts of l-aspartic acid, l-hydroxyproline, or l-proline to cultures of ova. In nine-cell ova a certain number of the blastomeres are destined to differentiate at a later period to form the embryonic plate, while those at the periphery are the first to be effected and probably differentiate into the trophoblast.

Peripheral flattening of blastomeres is an apparent reflection of the changing chemistry in newly differentiating cells. In the experiments performed with nine-cell ova, blastomeres have been caused to differentiate by changing their metabolic equilibrium by supplying amino acids which are undoubtedly indispensable to normal cellular differentiation.

The rôle of the zona pellucida in providing a physical force which might condition differentiation, evidenced as flattening of peripheral blastomeres, is apparently negligible since differentiation may be chemically induced before the entire perivitelline is consumed by an expanding morula (Figure 4, A).

Apparently these naturally-occurring amino acids do not alter the predetermined course of differentiation, but as Hammett (4) puts it: "The utilization by a cell, of l-proline or its homologue l-hydroxyproline or a common intermediary, are the chemical mechanisms by

which differentiation is expressed." The identity of the cells formed in the nine-cell ova of rabbit *RA*15 has not been established (Figure 4, A).

By the twentieth hour of culture, the trophoblast and inner cell mass had become clearly defined by the formation of the crescentric blastocyst cavity. From this time on, the cells of the trophoblast are more actively proliferating than are those of the inner cell mass. It was invariably from the cells of the trophoblast that newly differentiated cells formed when ova were exposed to l-proline and l-hydroxyproline (Figures 2, F; 3, A, B, C, D, H). This was confirmed by sections.

In normal development, the trophoblastic cells are the first to differentiate into hematopoietic anlage in the form of the early blood islands. Experimental ova, after three days of exposure to l-hydroxyproline and l-proline, showed newly differentiated cells which appeared to be of erythropoietic variety together with small round cells which were interpreted as being more highly differentiated cells of the same order. After five days of culture in the presence of these substances, the number of large round cells decreased while the number of small round cells increased, indicating maturation of the large round cell variety (Figure 3, B and Figure 5, C).

Both l-proline and l-hydroxyproline enhance differentiation expression, however l-hydroxyproline has the added effect of stimulating proliferation. This has been determined for Obelia Geniculata (5) and Drosophila (6) and the photographs show it is also the case with mammalian ova. Experimental ova cultured in l-hydroxyproline show a relatively greater number of recently differentiated cells than do those cultured in the same molar concentration of l-proline. This indicates that the compounds of intermediary metabolism of l-hydroxyproline probably differ from those formed in the metabolism of proline. It has been shown that the breakdown of beta-hydroxy-alpha-amino acids may differ from that of simple amino acids (Knoop, 7). Hammett (5) has postulated that this proliferation-enhancing property of l-hydroxyproline may be associated with a possible interchangeability OH and SH in intermediary metabolism. Addition of proper amounts of cysteine to cultures containing fertilized ova causes an acceleration of the cleavage rate.

Injections of l-proline into a tumor strain of mice results in the appearance of a larger number of tumors than normally occur and in a shorter time interval (8).

Proper concentrations of l-aspartic acid also favor precocious differentiation of blastomeres and Obelia Geniculata (9) and so resembles proline. The effect of proline is constantly limited to the cells of the trophoblast and inner cell mass. In addition the cells seen as a result of l-aspartic acid differ in their nuclear pattern from those resulting from l-hydroxyproline (Figure 3, E, H). In the former case multinuclear cells are often seen. The nucleus is limited by a well defined membrane and is of a vesicular nature. In the latter case the nuclei tend to be condensed and more closely resemble the nuclear pattern of adult white blood cells.

From these observations it appeared that l-proline and l-hydroxyproline were concerned with differentiation of cells resembling blood cells. It is interesting that both l-hydroxyproline and l-aspartic acid occur in high concentration in liver extract (10).

Clinical application in pernicious anemia of l-hydroxyproline and l-aspartic acid was tried. As is well known, there is difficulty in obtaining suitable cases. Most patients have complications of one kind or another, most have been treated with liver and most clinicians agree that cases of the "old fashioned primary pernicious anemia" are few and far between. Of seven cases of anemia that received treatment only two seemed to be typical enough for evaluation. Nevertheless, the results in all were similar and consisted of a prompt rise in reticulocyte counts from 0.5 per cent to 5, 10, and even 12 per cent, which lasted from three days to ten and then fell. There was no maintenance of the rise and the total red blood cell count was very little influenced. Subsequent liver treatment provoked the usual response in three of the seven cases.

l-Hydroxyproline was given subcutaneously in daily doses of 2 cc. of a 0.38 molar solution for from two to four weeks. l-Aspartic acid was given subcutaneously in 2 cc. doses of 0.45 molar concentration as the monosodium salt. Equal parts of both in a mixture were also given. The l-hydroxyproline was the more effective; l-aspartic having little or no effect. It seems that neither of these two are competitors of liver extract.[2]

Summary and Conclusions

Rabbit ova, in hanging drop and circulating cultures, were used as

[2]Appreciation is expressed to Drs. Hobart A. Reimann and L. Tocantins of Jefferson Hospital, Philadelphia, for their trial of these compounds in anemia.

test objects for the effects of l-proline, l-hydroxyproline, and l-aspartic acid in M/10,000, M/100,000, and M/200,000 concentrations in rabbit sera.

l-Proline enhances differentiation expression.

l-Hydroxyproline enhances differentiation expression and also stimulates proliferation.

l-Aspartic acid favors differentiation.

Certain cells appeared in the test ova earlier than in the controls. They looked like hematocytoblasts and so liver extract was used as a sort of control. The results were indistinguishable from those obtained with proline and hydroxyproline.

l-Hydroxyproline and l-aspartic acid injected subcutaneously in patients with "primary" anemia failed to compete successfully with liver extract.

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Growth, 1941, **5**, 351-358.

THE GROWTH REPRODUCTION CYCLE: II

H. L. EICHHORN

3459 West 65th Street, Chicago, Illinois
(Received for publication, August 11, 1941)

In the effective development of any animate or inanimate organism —whether through omnipotence, human intelligence, trial and error or natural selection—there must appear a basic plan of operation. This plan has ordinarily nothing to do with dimension or specific mechanism. It is abstract; having to do with overall principle in terms of force, movement, relative displacement, etc. Usually it is concerned in some accomplishment by most direct action, i.e., with least waste and consequent greatest efficiency. The time required for the unfolding of such a plan may be infinitesimal, a matter of moments, months, or millions of years.

If an observer seeks to become entirely informed regarding the organism, it is only logical that he should first learn the functional plan about which the organism has been developed. This involves a matter of perspective without distraction by specific details existing as consequence rather than as cause.

Some time ago (Vol. IV, p. 1), the writer attempted to discuss such a plan for the functions of growth and reproduction, using an abstraction which he referred to as a "living machine." It was shown that such a "machine" would trace a reproduction loop made up of four paths, the mathematical expressions of which were given (Expressions 14 to 17). The expression for the growth movement, when integrated, produced a mass-time relationship (Expression 25) more descriptive of actual animate growth than any expression previously presented. This was considered as evidence that the abstract paths which had been described were also closely identified with the movements of actual life.

The discussion of the paths was terminated at the end of a single reproduction loop on the assumption that the loop would prove to be an end in itself and, but for decreased efficiency due to "senescence," would be subject to immediate repetition. Repetition accompanied by decline had been observed with hens but the meaning of

this was not understood. It now appears that the decline toward ultimate maintenance level does not indicate decreased efficiency but is as logical, even in abstract consideration, as growth or the rise to reproduction level. The object now is to investigate the manner in which the ultimate maintenance level is reached.

Approach to Ultimate Maintenance Level

It is common knowledge that the commercial hen goes through a series of expanding and receding movements. Each complete change requires the time of one year. The figure so formed is here termed a reproduction loop. With the passing of each year, the hen moves a step nearer to ultimate maintenance level. The extent of this movement is commonly covered by saying that egg production decreases about 20 per cent per year. It seems evident then that the course to ultimate maintenance level in this case consists of a series of loops, decreasing in size and converging to a point where the value of the creative component is zero. Such a course is indicated in Figure 10.

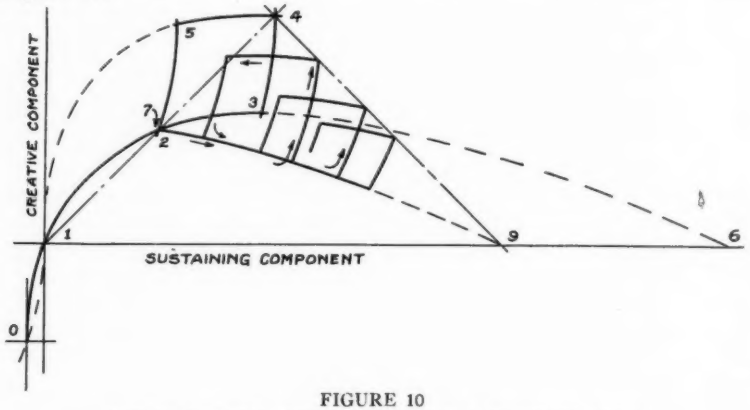

FIGURE 10
Converging Spiral Course to Ultimate Maintenance Level

A study of the time factor indicates that some mammals follow a similar course although several years may be required to complete each loop. In most of these cases, there is no common agreement as to when the level of pure maintenance is reached, leading to the supposition that the number of reproduction loops is not constant—that some of the loops are shunted by a final direct movement to ultimate maintenance level.

As opposed to birds or common mammals, the human being seems to present a case in which all loops except one have been shunted so that the movement to ultimate maintenance level is a direct path such as 7-9 in Figure 11. In this case the time when ultimate main-

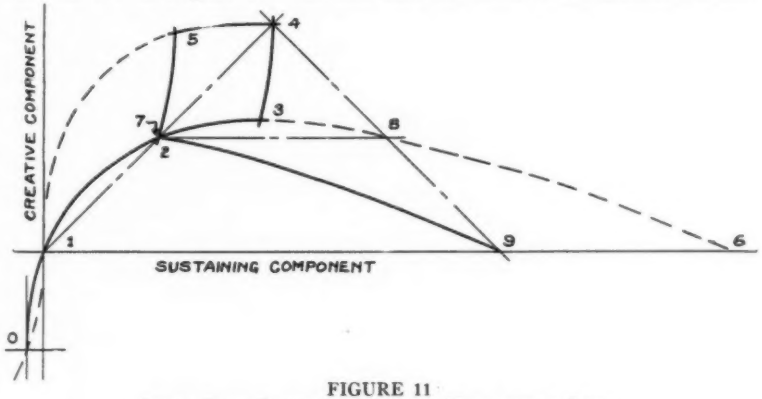

FIGURE 11
SINGLE PATH COURSE TO ULTIMATE MAINTENANCE LEVEL

tenance is reached (menopause) has a definite value. It now becomes evident that the term "cycle" should be applied not just to one loop but to the entire series of paths which repeat generation after generation.

The significance of the cycle may be clarified somewhat when it is considered to represent a progressive ideal capacity; while actual food consumption, actual growth, actual reproduction, etc., indicate the degree in which this ideal capacity is applied. Considered in this way, "vital power" (Vol. IV, page 2) becomes "capacity for vital power" or "vital power capacity." It is this capacity, apparently, which passes through the confines of the system during reproduction leaving as a reaction a negative movement from 4 to 7. This is an effect analogous to the effect of the first law of thermodynamics. The path 7-9 about to be described (or the converging spiral based on this path), is a move toward quiescent equilibrium—an effect analogous to the effect of the second law of thermodynamics.

In Figure 11, 0-1-2-3-4-5-7 represents the progenitor paths up to the completion of the reproduction loop as previously described and 0-1-2-3-6, the path of a "living machine" planned for growth without reproduction; 4-9, a line of constant F representing the limiting food

capacity reached during growth, is described by the expression $(c + s) = 4f$. The coördinates of Point 8, found by solving for the intersection of $(c + s) = 4f$ and Expression 14, are $c = f$ and $s = 3f$. The coördinates of Point 7 are $c = f$ and $s = f$ so that a horizontal line through 7 also passes through 8. Point 3 is the maximum on the path 0-1-2-3-6. Further movement beyond 3 along this path would have meant a decline toward ultimate maintenance level. If the progenitor (planned for reproduction) had not been diverted, the same interval of time used in going from 3 to 4 would have been used in going from 3 to 8 (expansion in food capacity in both cases being from $3f$ to $4f$). During the negative movement along 4-5-7, the path 8-6 may be considered to have been translated a distance of $(-2f)$ so that the path to ultimate maintenance level would be from 7 to 9 corresponding in rate of change to the path 8-6. It is to be noted that the path 7-9 intersects the level of pure maintenance at the limiting capacity line 4-9. The course then followed by a "living machine" of the single loop type from beginning of life to attainment of ultimate maintenance would be 0-1-2-3-4-5-7-9. Although the routes followed by the converging spiral and single loop types differ, their termination is the same—Point 9.

Collecting the F-values at the various junctures (Vol. IV, page 9),

$$(c + s)_2 = 2f \tag{26}$$
$$(c + s)_3 = 3f \tag{27}$$
$$(c + s)_4 = 4f \tag{28}$$
$$(c + s)_5 = 3f \tag{29}$$
$$(c + s)_7 = 2f \tag{30}$$
$$(c + s)_9 = 4f \tag{31}$$

Time Positions of Path Junctures

h equals time elapsed between start of major growth and attainment of mature weight or mature food capacity (Vol. IV, page 11). From Expressions (21) and (18),

$$(c + s) = \frac{4f}{h}\left(2A - \frac{A^2}{h}\right) \tag{32}$$

Time at which any point occurs after start of major growth may now be found by substituting for $(c + s)$ the corresponding values in terms of f. At Point 2 or 7,

$$2f = \frac{4f}{h}\left(2A - \frac{A^2}{h}\right)$$
$$A = (1 \pm \sqrt{1/2})h$$

$A = (1 - \sqrt{1/2})h$ applies to end of path 1-2, designated as Point 2. $A = (1 + \sqrt{1/2})h$ applies to end of path 5-2, designated as Point 7. The values of A for Points 3, 4, and 5 may be found by the same method, so that,

$$A_2 = (1 - \sqrt{1/2})h \tag{33}$$
$$A_3 = 1/2h \tag{34}$$
$$A_4 = h \tag{35}$$
$$A_5 = 1\tfrac{1}{2}h \tag{36}$$
$$A_7 = (1 + \sqrt{1/2})h \tag{37}$$

Time elapsed between Point 7 and Point 9 for the single loop type is same as between Point 2 and Point 4 (expansion in capacity in both cases being from $2f$ to $4f$). Total time elapsed at Point 9 is occurrence time at Point 7 plus time elapsed between Point 7 and Point 9 or

$$A_9 = A_7 + (A_4 - A_2) = (1 + \sqrt{1/2})h + h - (1 - \sqrt{1/2})h = (1 + 2\sqrt{1/2})h \tag{38}$$

Significance of Path Junctures

It has been demonstrated (Brody, 1927) that puberty occurs at the inflection point of observed growth curves. The determination of the inflection point should therefore be an important test for any growth expression. The inflection point may be defined in purely mathematical terms. It is the point at which the second derivative equals zero. The second derivative of Expression 25 does equal zero when $A = (1 - \sqrt{1/2})h$. Since $(1 - \sqrt{1/2})h$ is the age at which Point 2 is reached, Point 2 must definitely represent the age of puberty. Puberty may therefore be concisely defined as the point at which the growth movement first touches the reproduction loop.

Point 3 has already been designated as the optimum point for start of reproduction and Point 4 as the point of attainment of mature weight and mature food capacity (Vol. IV, page 3). Point 5 represents start of decline of reproductive activity and Point 7, the swing toward ultimate maintenance level. Point 9 evidently represents menopause. For convenience we will now refer to the junctures (3), (4), and (5) as optimum inception, capacity attainment and recession.

Time Application

Expressions (33) to (38) present the times at which various junctures are reached in terms of h. The value of h for any case may be determined when the time occurrences of any two junctures or the time interval between them is known. Several examples will now be given.

A hen of the Leghorn class is "designed" to complete a single reproduction loop of its converging spiral in just one year.

$$A_7 - A_2 = (1 + \sqrt{1/2})h - (1 - \sqrt{1/2})h =$$
$$1 \text{ year} = 52.18 \text{ weeks} \qquad (33), (37)$$
$$h = 36.9 \text{ weeks}$$

The loop junctures then occur as follows:

$A_2 = (1 - \sqrt{1/2})h = 10.8$ weeks—puberty.
$A_3 = 1/2\,h = 18.45$ weeks—optimum inception.
$A_4 = h = 36.9$ weeks—capacity attainment.
$A_5 = 1\frac{1}{2}h = 55.35$ weeks—recession.
$A_7 = (1 + \sqrt{1/2})h = 63$ weeks—first reproduction loop completed.

These figures will appear very familiar to any one who has had practical experience with Leghorn hens. They may be further adjusted to allow for the short period of time (about three days) between start of major growth and time of hatching (Figures 6 and 7).

It must be remembered, however, that the cycle is made up of paths considered to be ideally perfect. We may give to each of several machinists a job of making a steel block of certain shape and size. After the pieces are made, the individual blocks will be characterized not by the dimensions given to the machinists but by the individual variations from those dimensions. Similarly a breed may be described by referring to the variations between the course followed by that breed and the ideal cycle paths, and an individual of that breed may be described by referring to its individual variations from the paths ordinarily followed by the breed.

This point might be illustrated by referring to another breed of hens—the Plymouth Rock. The Leghorn and the Rock are both very desirable breeds but the paths followed by them do not coincide. In the case of the Rock, Junctures 2, 3 and 4 are reached at later times than in the case of the Leghorn. Therefore, when Point 4 has been reached, less of the year remains for completion of the

reproduction loop. Consequently we may expect a shortened path 4-5 and a distorted path 5-7. Where high reproduction is the desired purpose, as in the case of the Leghorn, the ideal paths and tempo should apparently be closely followed. For another purpose, certain definite variations are required. These variations may be placed in time and in degree by reference to the basic ideal cycle.

As an example from another group, consider an individual from a herd of dairy cattle where major growth is taken to start three months before birth (Vol. IV, page 15) and puberty to occur at the age of 10 months or 13 months after start of major growth.

$A_2 = (1 - \sqrt{1/2})h = 13.$

$h = 44.4.$

$A_2 - 3 = (1 - \sqrt{1/2})h - 3 = 10$ months—puberty.

$A_3 - 3 = 1/2\,h - 3 = 19.2$ mo. = 1 yr., 7.2 mo.—optimum inception.

$A_4 - 3 = h - 3 = 41.4$ mo. = 3 yrs., 5.4 mo.—capacity attainment.

$A_5 - 3 = 1\frac{1}{2}h - 3 = 63.6$ mo. = 5 yrs., 3.6 mo.—recession.

$A_7 - 3 = (1 + \sqrt{1/2})h - 3 = 75.8$ mo. = 6 yrs., 3.8 mo. first loop completed.

As is the case with hens, the breeders have it within their power to vary this proportion for desired special characteristics.

In the first example, birth occurred at about start of major growth; in the second, birth was retarded. The human being presents an example where birth is advanced by a number of years or, conversely, where minor growth is extended beyond birth for a number of years. For this example, consider puberty at approximately 12 years of age and menopause at 50.

$$A_9 - A_2 = (1 + 2\sqrt{1/2})h - (1 - \sqrt{1/2})h = 50 - 12$$

$$h = 17.9$$

$$A_2 = (1 - \sqrt{1/2})h = 5.25$$

Therefore, in this case, A_2 occurred 5.25 years after start of major growth or major growth began (12 — 5.25) or 6.75 years after birth.

$A_2 + 6.75 = (1 - \sqrt{1/2})h + 6.75 = 12$ yrs.—puberty.

$A_3 + 6.75 = 1/2\,h + 6.75 = 15.7$ yrs.—optimum inception.

$A_4 + 6.75 = h + 6.75 = 24.65$ yrs.—capacity attainment.

$A_5 + 6.75 = 1\frac{1}{2}h + 6.75 = 33.6$ yrs.—recession.

$A_9 + 6.75 = (1 + 2\sqrt{1/2})h + 6.75 = 50$ yrs.—menopause.

It is evident from Figure 9 that the ideal cycle is subject to con-

siderable distortion in the case of man. Just how this affects computed time of path junctures cannot be said at present.

Summary

The growth-reproduction cycle is again considered and the extension of the course to ultimate maintenance level is described. The expression for rate of movement is applied to develop the significance of path junctures and their time positions.

Growth, 1941, **5**, 359-364.

DIFFERENTIAL GROWTH AND MORPHOLOGICAL PATTERN IN DAPHNIA

A. H. HERSH AND B. G. ANDERSON[1]

Biological Laboratory, Western Reserve University, Cleveland, Ohio
(Received for publication, August 20, 1941)

During the development of the organism from the small simple-patterned spherical egg to the many times larger adult with its complicated pattern there are changing size relations in the various dimensions of the whole and of the parts until the organism reaches its final size.

This process of differential growth has been frequently studied by means of the relative growth function,

$$y = bx^k$$

in which x and y are the variable measurements and b and k are constants. The morphological patterns of most animals are so complicated that the relative growth function is quite inadequate to specify all the changing details of the complicated pattern.

It seems desirable to make a comparison of two species of a relatively less complicated organism so far as the data allow to show something of the main results that can be obtained by the use of the relative growth equation. Such an organism is Daphnia for which two species have been studied from the first instar to the full grown adult for certain measurements which serve to specify the main dimensions of the relatively simple pattern (Anderson, 1932; Anderson, Lumer and Zupancic, 1937).

The main points can be discussed with regard to the accompanying Figure 1. The two major dimensions, total length (x) and total height (y), of *Daphnia magna* from the first to the thirteenth instar and from the first to the twentieth instar for the smaller species *D. pulex* are given in Table 1 and plotted logarithmically in conformity with the relative growth equation.

The phase of growth from the egg to the first instar in each species is indicated in Figure 1 by the dashed lines to the left of the solid

[1]Now at The Department of Botany and Zoölogy, West Virginia University, Morgantown, W. Va.

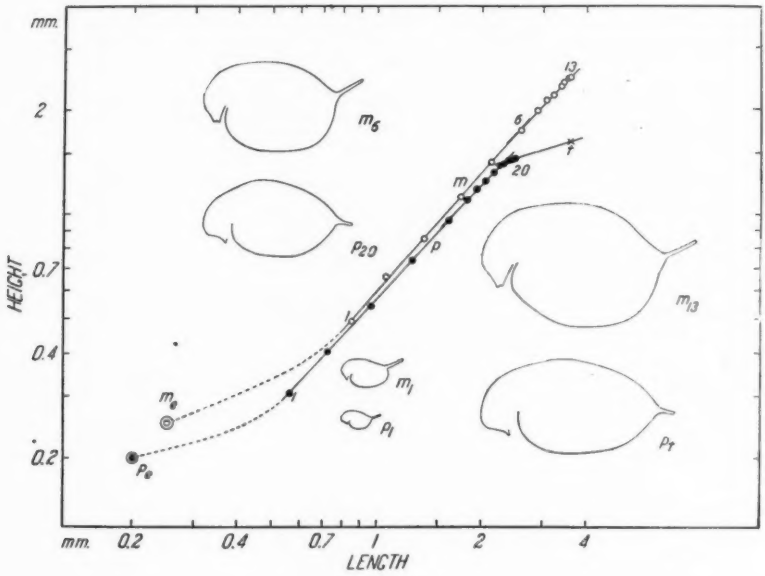

FIGURE 1

Logarithmic plot of height (y) against length (x) for *Daphnia magna* and *D. pulex*, with the outlines of the animals shown at the instars indicated by the subscripts. See text for further discussion.

lines which represent the actual data. The egg of *D. magna* is 0.25 mm. in diameter; that of *D. pulex* is 0.20 mm. in diameter. Since the egg is spherical it is obvious that during the embryonic period of growth the length increased faster than the height, and if the embryonic cells are approximately the same size within each species, then the further inference may be drawn that the planes of successive cell divisions were more frequently at right angles to the long axis of the developing animal rather than parallel to it.

If the changing size relations before the first instar conformed to the relative growth equation it is clear that the values of k would be low and be about the same for both species, but an estimate of the values cannot be given at present because of lack of data on the embryonic measurements. In any case it would seem that there would be a more or less distinct break or sharp change in the slope of the curves before the first instar.

TABLE 1

Averages in mm. of the total length (x) and height (y) for *Daphnia pulex* (51 animals) and *D. magna* (7 animals) at each instar, with values of b and k for the several epochs. (The values of b in the case of *pulex* are to replace the incorrect values as published by Anderson, *et al.*, 1937. Table 2, page 452.)

Instar	Pulex Length	Pulex Height	Allometric constants	Magna Length	Magna Height	Allometric constants
1	0.566	0.306		0.85	0.49	
2	0.721	0.402	$k = 1.09$	1.06	0.66	
3	0.961	0.543	$b = 0.569$	1.37	0.85	$k = 1.13$
4	1.265	0.735		1.75	1.12	$b = 0.60$
5	1.608	0.960		2.13	1.42	
6	1.813	1.102		2.59	1.75	
7	1.933	1.178		2.88	1.99	$k = 1.05$
8	2.057	1.245	$k = 1.03$	3.06	2.12	$b = 0.65$
9	2.167	1.321	$b = 0.593$	3.21	2.22	
10	2.247	1.374		3.39	2.34	
11	2.306	1.398		3.45	2.41	
12	2.340	1.413		3.53	2.45	
13	2.370	1.423		3.60	2.48	
14	2.385	1.426				
15	2.403	1.427	$k = 0.315$			
16	2.422	1.427	$b = 1.08$			
17	2.446	1.434				
18	2.460	1.434				
19	2.484	1.444				
20	2.489	1.443				

After the embryonic period from the first preadult instar to the first adult instar (the sixth in *magna* and the fifth in *pulex*) the values of k for the two species are 1.13 for *magna* and 1.09 for *pulex*. In each species the height is increasing faster than the length, slightly more so in *magna* than in *pulex*.

If the line for *magna* in Figure 1 were extended downward, it would pass through the point representing the first instar of *pulex*, and the height and length for the two species would be the same. But the total pattern is very incompletely specified by these two measurements. The more detailed differences can be seen from the outlines in Figure 1 and marked m_1 and p_1. There is a greater bulge below the caudal spine in *magna* than in *pulex;* there are differences in the shape and carriage of the head as well as smaller differences in general outline. These smaller differences at this stage and at all later stages give, in general, a more plump appearance to *magna* than to *pulex*. This contrast in the two species becomes more marked at the size of *magna* at the sixth instar. By this time *pulex* has reached its maxi-

mum size and has passed through 20 instars (see Figure 1, m_6 and p_{20}).

After the first adult instar the value of k in *magna* changes from 1.13 to 1.05 and in *pulex* from 1.09 to 1.03. In *pulex* there is a further change in k at the thirteenth instar from 1.03 to 0.315. The tendency for *magna* to increase more in height than *pulex* persists throughout. If the length of *pulex* were increased to the size of *magna* at the thirteenth instar, it is clear that in form *pulex* would seem somewhat attentuated as a result of the continuation of these differences in relative growth. (Compare m_{13} and p_t in Figure 1).

As instances accumulate on the application of the relative growth equation to data on differential growth of various organisms, it is clear that the whole course of growth can be subdivided into epochs marked by changes in the numerical values of the constants b and k, but it is also clear that such epochs need to be brought into relation to the time course of growth of the elements x and y. In *D. magna* there are at least three and in *D. pulex* there are perhaps four such epochs. But the entire course of the relative growth curves, especially in *D. pulex*, is similar to the consequences for relative growth derived algebraically from the sigmoid time course of x and y by Lumer (1937).

The changes in k have already been discussed. The constant b is the value of y when $x = 1$, and in most cases represents an extrapolation to the left, but such is not the case here since the original micrometer units have been converted to millimeters. The value $x = 1$ for *magna* is very close to the length of the organism at the first instar and for *pulex* it is the approximate length of the animal at the third instar. Consequently for the embryonic period the numerical value of b in the equation represents an extrapolation to the right of the data for both species. But for the adult epochs the value of b represents an extrapolation to the left as in the usual case. For the preadult stage of growth the value of b falls within the range of the data (see Lumer, Anderson and Hersh, Amer. Nat., in press); b in all cases gives the approximate value of the height for all epochs of growth in both *magna* and *pulex* referred to the length of *magna* at the first preadult instar, or of *pulex* at the third preadult instar.

In relative growth studies there is always the question of the relation between ontogenetic relative growth and the differences in the adult morphological pattern between related species, i.e., evolutionary

relative growth. It has been found for a few types of organisms that when x and y are homologous measurements of adults from different but related species, the data likewise conform to the relative growth equation. That is, the equation is used to define allometric tribes. Such tribes have not as yet been studied in the Cladocera, but the problem in regard to all such tribes is that of the relation between the b and k for the allometric tribe and the b and k for the ontogenetic relative growth of the representative members of the allometric tribes. Do the values coincide, or in what way do they differ? If the two species of Daphnia belong to the same allometric tribe, it is clear that for the height/length relation, the values of b and k for the tribe would differ from the ontogenetic values of both *D. magna* and *D. pulex*, which in turn differ from each other. On the graph the line for the allometric tribe would pass through the last points indicated for *magna* and *pulex*. It is immediately clear from the graph that the two species reach their final adult proportions over different ontogenetic paths, but perhaps belong to the same allometric tribe.

This difference is no doubt related to the relative number of cell divisions along the longitudinal axis and the height axis, and also to the direction of cell enlargement. In both species the carapace cells are more or less square, but in *magna* they are arranged at right angles and in *pulex* at an angle of approximately 45° to the longitudinal axis. This difference as well as the differences in the ontogenetic b's and k's for the several epochs is an expression of the manner in which the genetic differences between the two species act to bring about their characteristic effects on the morphological pattern.

Summary

In *Daphnia magna* and *D. pulex* the egg is spherical, but at the first instar the length (x) of the animal is already much greater than the height (y). The dimensions conform to the relative growth equation, $y = bx^k$, with different values of the constants after the first instar than before. The height increases relatively faster than the length, slightly more so in *magna* than in *pulex*. The two species reach their final adult proportions over different ontogenetic paths, but perhaps belong to the same allometric tribe.

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Growth, 1941, **5**, 365-371.

TRUNK SIZE: A SURVEY OF METHODS TOGETHER WITH NEW CONVERSION EQUIVALENTS*

H. GRAY

Stanford University School of Medicine, San Francisco, California
(Received for publication, August 25, 1941)

Surveys of rival methods usually exhibit the humanity of authors by ending with an *ex cathedra* verdict on the one and only technique to be used by all sensible men. Such a verdict would be less often ignored by the next expert dealing with the particular physical trait, if supported by parallel data on the same subjects measured by the rival methods. The purpose of this paper is to present such data for measurements on the human trunk.

MATERIAL

The subjects were male adult convicts between the ages of 20 and 60 years, averaging 33 years, measured at the Illinois State Penitentiary in Joliet from 1927 to 1929. Any appearing deformed or diseased were omitted. The number used for this study were 166 whites and 97 negroes. The data were treated separately for the two races. The average stature was 1,693 mm. for the whites, 1,716 mm. for the negroes. The difference is worth notice, because it accounts at least in part for the differences which will be noticed later in the racial averages for the traits to be studied below. For it has been pointed out that: "A coefficient showing the relationship between stature and the deviation of the components (sitting height, leg length, etc.) from their probable value shows that . . . in tall individuals sitting height forms a relatively smaller, and leg length (*S* minus *Si*) a relatively larger proportion of total stature than in short ones" (Harris). The size of the traits relative to stature could easily be compared in the two races, but will not be treated in the present paper.

TRUNK LENGTHS

The averages and variabilities are shown in Table 1, arranged in

*Aided by the Rockefeller Fluid Research Fund.

TABLE 1
TRUNK-LENGTH CONSTANTS

Length		Race	Mean	± *pe*	Standard Deviation	Critical Ratio, *Diff./pe*
Sitting-height	*Si*	*W*	904	1.7	31.6	
		N	882	2.4	33.0	
		W — N	22			8
Upper measurement	*v-sy*	*W*	846	1.7	32.6	
		N	821	2.3	32.6	
		W — N	25			9
Trunk-height	*sst-seat*	*W*	586	1.1	24.6	
		N	572	1.5	25.6	
		W — N	14			8
Ant. trunk-length	*sst-sy*	*W*	518	1.3	24.9	
		N	499	1.6	23.5	
		W — N	19			9
Abdom. length	*xi-sy*	*W*	363	1.2	22.9	
		N	348	1.5	21.4	
		W — N	15			8
Lower Abd.	*epi-sy*	*W*	194	0.94	17.9	
		N	190	1.17	17.0	
		W — N	4			3
Upper Abd.	*xi-epi*	*W*	168	1.0	19.2	
		N	160	1.2	18.0	
		W — N	8			5
Chest length	*sst-xi*	*W*	156	0.66	12.6	
		N	151	0.87	12.7	
		W — N	5			5

order of size. Also the difference in millimeters between the means for the two races are noted, and are shown to be significantly different whenever the value of the critical ratio (difference ÷ probable error of the difference) in the last column is 3 or more.

Sitting-Height, Si

As a measure of trunk-length this has been the commonest in anthropological literature, by far. Surveys of different methods for this item have been published by several writers, some contributing personal opinions (Gray and Root 1921, Gray 1923, Ciocco 1937), some contributing personal evidence. For example, it was shown for 114 schoolboys (Gray, 1922) that stem-length (subject sitting on floor) was regularly less than sitting-height (subject sitting on box) by usual method, by 2 per cent of the sitting-height at the age of 6 years, by 1 per cent from 7 to 12 years, and about 3 per cent

from 13 to 18 years, agreeing well with 3 per cent for adults reported by Walker (1916).

Upper Measurement, v-sy

This dimension has been little used by physical anthropologists, but fairly frequently by endocrinologists. The motive has been its apparent simplicity in that the upper measurement has been supposed to be equal to the lower measurement, *sy*, the height of the top of the pubic symphysis from the floor. The dimension will be studied here although it is open to several objections:

1. Anthropological norms are scanty (as compared with sitting-height norms).

2. The supposition of equality in healthy persons has been proved by nobody, and frequently is false, particularly when the age of the subject varies, as it often does. Even in our adults and considering only the averages,

	White	*Negro*
Upper measurement	846	821
Lower measurement	847	896

inequality is severe for the negroes, 75 mm/821 = say 9+ per cent. And furthermore, for the whites, though the two averages agree nicely, individual subjects show inequalities which are not only frequent, but marked, and not always in the same direction; e.g., in 10 white men at random the difference *vsy* minus *sy* was not zero but —41, —66, 17, —2, —63, 38, —78, —77, 12, —68 mm.

3. The pubis is so movable a landmark, with relatively slight differences in posture, that it easily produces errors in measurement, as has been demonstrated (Gray, 1923) both from data reported by others, and from our experience.

Trunk-Height of Wertheimer, sst-seat

This is an unusual choice of landmarks.

Anterior Trunk Length, sst-sy

This dimension, though not very widely used, deserves attention largely because of its applicability alike to man and to other animals (Schultz). Its widest use in man has been by the Italian school, who have tried to refine it by division into three parts:

Thoracic length	= Supra sternal notch to base of xiphoid	= *sst — xi*
Upper abdominal length	= Xiphoid to epigastric point (at level of costal margins)	= *xi — epi*
Lower abdominal length	= Epigastric point to top of symphysis	= *epi — sy*

Conversion by Regression Equations. So-called statistical constants, like the averages tabulated above, do not reveal the relationship between any chosen pair of dimensions; the two-way tabulations are bulky; but, since they all appeared approximately linear, i.e., of the form

$$Y = \overline{Y} + b(X - \overline{X}),$$

we show in Table 2 the essential statistics useful for any future ob-

TABLE 2
TRUNK-LENGTH REGRESSIONS

Y	X	Race	Regr. Coef. b	r	s_E	s_b
1	2	3	4	5	6	7
Sitting-height	Vertex-symphyses	White	0.8995	.9267	11.88	.0283
		Negro	0.9466	.9340	11.80	.0368
Sitting-height	Supraster-nale-seat	White	1.1300	.8783	15.12	.0477
		Negro	1.1719	.9088	13.79	.0546
Sitting-height	Supraster-nale-symphysis	White	0.9492	.7485	20.97	.0653
		Negro	1.0950	.7793	20.70	.0894
Sitting-height	Supraster-nale-xiphoid	White	1.4226	.5675	26.04	.1602
		Negro	1.1051	.4263	29.89	.2381
Sitting-height	Supraster-nale to epi-gastric point	White	0.7826	.5163	27.08	.1007
		Negro	1.0688	.6260	25.77	.1352

servers interested in converting X, a physical anthropometric trait measured by one method, to Y, the probable value expected by another method preferred by the later investigator. The notation used in the various columns is as follows:

(1) Y, the preferred dimension, to be estimated by conversion from X.

(2) X, the measured dimension.

(3) Race.

(4) b, the regression coefficient of Y on X.

(5) r, the Pearsonian coefficient of correlation.

(6) s_E, the standard error of estimate, i.e., $s_Y \cdot \sqrt{1 - r^2}$, where s_Y is the standard deviation of the Y's, using five digits in computing, not merely the three digits shown in Table 1.

(7) s_b, the standard error of the regression coefficient b, i.e., $s_E \div \sqrt{\Sigma(X - \overline{X})^2}$.

The equation, for example, to estimate sitting-height Y or E when the observed "upper measurement" (vertex-symphysis) X was recorded as 840 millimeters, will be:

$E = \overline{Y} + b(X - \overline{X})$.
$= 904 + 0.8995\ (840 - 846)$.
$= 904 - 5.4$.
$= 899$.

The necessary sampling errors will be:

The standard error estimate, $s_E = 11.88$.

The standard error of the ordinate $\overline{Y}$ is not $= s$ (the standard deviation of Y) $/\sqrt{N} = 31.6\ /\sqrt{166} = 2.45$; but is $s_E\ /\sqrt{N} = 11.88\ /\sqrt{166} = 0.922$.

The standard error of the regression coefficient, $s_b = 0.0283$.

Suppose an observer has measured a fresh sample and has obtained an upper measurement of 840 mm. and a sitting height of 909 mm., and wishes to judge whether this fresh sample is significantly different from the value E expected from the equation.

(*a*) When the sample consists of a single individual, we have the *diff.* $Y - E = 909 - 899 = 10$ mm., with $SD_{diff.} = s_E = 11.9$, *diff./SD* $= 0.84$; and entering probability table we find the tail $= 0.20$, i.e., a chance of only 1 in 5 trials, which is not significant.

(*b*) When the two fresh items represent respective means on a sample of say 100 individuals, we have the *diff.* $Y - E =$ again 10 mm., but this time with *SD* of $s_E/\sqrt{100} = 1.19$, *diff./SD* $= 8.4$, which is plainly significant; in other words, the equation is not suitable to express the relationship of the two characteristics in the fresh sample.

Trunk Diameters

Transverse Trunk Diameters

For each of our subjects we have measured the following widths at different levels of the trunk: (1) Bi-acromial shoulder diameter (*BA*), (2) The usual transverse chest diameter at level of 4th costo-chondral cartilage (*T*), (3) The hypochondriac transverse of the Italian school (which we denote as *HT*), and (4) the bi-cristal diameter of the pelvis (*BC*).

Trunk Depth

We have here the measurement at the usual level (but with the less usual curved compass) discussed in our earlier (1935) paper as

D, and also the unusual thoracic-abdominal level, which we here denote as *HD*.

The averages and other principal constants are shown in Table 3.

TABLE 3
TRUNK-DIAMETER CONSTANTS

Diameter		Race	Mean	±*pe*	Standard Deviation	Critical Ratio
Bi-acromial	*BA*	*W*	387	1.0	18.1	
		N	396	1.3	18.8	
		W — *N*	9			5.6
Transverse chest	*T*	*W*	336	1.1	20.4	
		N	342	1.6	22.2	
		W — *N*	6			3.1
Hypochondriac transverse	*HT*	*W*	289	1.0	18.5	
		N	285	1.1	15.6	
		W — *N*	4			2.3
Bi-cristal	*BC*	*W*	285	0.8	15.5	
		N	268	1.1	15.4	
		W — *N*	17			12.8
Depth of chest	*D*	*W*	206	0.8	15.8	
		N	201	1.1	15.1	
		W — *N*	5			3.8
Hypochondriac depth	*HD*	*W*	201	1.1	20.4	
		N	205	1.4	19.8	
		W — *N*	4			2.2

The means and other statistics differ slightly from those reported in a prior paper because the hypochondriac dimensions were taken on only a portion of that series and therefore represent somewhat smaller numbers of men.

TABLE 4
TRUNK-LENGTH REGRESSIONS

Y	*X*	Race	Regr. Coef. *b*	*r*	s_E	s_b
1	2	3	4	5	6	7
Chest transverse	Hypochondriac transverse	White	0.8014	.7269	13.99	.0588
T	*HT*	Negro	0.9666	.6795	16.28	.1060
Chest depth	Hypochondriac depth	White	0.4443	.5738	12.95	.0492
D	*HD*	Negro	0.5668	.7417	10.14	.0520
Bi-acromial	Transverse	White	0.4907	.5521	15.09	.0575
BA	*T*	Negro	0.7814	.9240	7.17	.0328
Bi-cristal	Bi-acromial	White	0.4358	.5104	13.29	.0570
BC	*BA*	Negro	0.3648	.4436	13.83	.0749
Bi-cristal	Transverse	White	0.1722	.2269	15.05	.0574
BC		Negro	0.2970	.4271	13.95	.0638

Each pair of measurements was plotted. The distributions looked satisfactorily linear, and yielded as before, the regression data shown in Table 4.

Summary

Similar but not identical methods of measurement have been a persistent problem in the study of body-build. The recent literature of physical anthropology affords some critical surveys. As a practical supplement, this paper presents, for each of the commoner trunk-lengths and breadths, measurements by two (occasionally more) rival methods; together with regressions which permit translation of a measurement reported by one method to the value which would probably have been secured if the dimension had been measured by a preferred method. Thus many dormant data can be revivified for comparison with more recent observations. A similar study of leg-lengths, which are both more complex and more important clinically, as in orthopedics, will be shown elsewhere.

I am indebted to Miss Sylvia Lowe for extensive assistance.

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Growth, 1941, **5**, 373-383.

LEG-LENGTH: A SURVEY OF METHODS TOGETHER WITH NEW CONVERSION EQUIVALENTS*

H. GRAY

Stanford University School of Medicine, San Francisco, California
(Received for publication, August 25, 1941)

ASPECTS OF BODY-BUILD

The fascination of the human figure has been unfailing through the ages, and still is to observers of all ages (after adolescence). The angle of observation is commonly esthetic, and—so far as emotion permits of reason—a fine figure is always accounted to be an asset. From the angle of anatomy, however, the fine figure of classic Greek art often reveals the intersexual; and furthermore from the angle of function has recently been found by Bayer (1940) in this clinic, to implicate intermediacy, sometimes even inferiority. Her findings may well be a source of keen consolation to that great majority who cavil about their God-given curves.

From the angle of constitution (diseases of the body, brain, or behavior) we adduce two medical authorities: first, an experimental geneticist, Stockard (1938) who wrote that "Structural disharmonies and deviations in size call forth somewhat modified states in the nervous system of the animal so that these conditions become of definite interest in the study of nervous and mental diseases"; and secondly, a clinician, Thomas Lewis (1934) who urged us to "Realize clean-cut facts, for however trivial a fact may seem at first sight, there is none that is infertile." This much is addressed to those who doubt the utility of all those monotonous measurements.

The medical study of build is plainly dependent on the clean-cut measurements in good use among physical anthropologists. In clinical medicine, however, history is repeated tiresomely: an index of build is published by some ingenious person, sometimes is supported by others, sometimes is claimed to be superior to existing indices, sometimes even receives international vogue, though seldom for as long as a decade. Fairly rigorous comparisons are occasionally made,

*Aided by the Rockefeller Fluid Research Fund.

but not often enough to discard the obsolete and clarify the valid standards of measurement, let alone to consider efforts at standardization under two heads: unification and conversion-equivalents.

Unification

National and international compromises, to achieve periodical discard of inferior technique and agreement on preferred dimensions, have been urged increasingly in recent years. Hrdlicka (1937) emphasized the value of historical review as the only basis on which anthropometry can reach satisfactory status; and has lately given space in his journal to a number of articles along this line (references collected in bibliography under title: Unification).

Conversion Equivalents

Despite all such historical reviews, many competitive methods are current, even in the same country. Even if a miracle of uniformity were achieved tomorrow, the literature would remain replete with observations useless for comparison until converted to the probable values had the item been measured by the preferred method. For such conversion the obvious though dull task is the calculation of equivalents. Such a quantitative comparison of parallel values obtained by somewhat similar methods can reveal significant differences; for example, as was recently pointed out in a paper on chest-depth in 246 males, an error of the order of 10 per cent can be avoided by using regressions to translate the less favored technique (in that case the curved spreading compass) to the value most probable had the measurement been made with the preferred technique (in that case the sliding caliper). The necessary regressions and a simple conversion table were given. This task, so obvious to us, has been privately condemned as follows: "No one who has any experience with the two methods and has the instruments would be likely to use the spreading in preference to the sliding compass." We feel obliged to insist: (*a*) The eventual importance of analysis of parallel data is naturally weighed differently by those interested in a given trait, and those disinterested; but the principle of the desirability of such analyses has been repeatedly encouraged even by many who have themselves neglected the chore of recording evidence with regard to their own precision of measurement. (*b*) Since several well-known anthropologists, cited in that paper, have in fact used the

spreading compass, the condemnation may be termed an authoritarianism not conducive to maximum utilization of authentic data.

Observations in the literature must then be scanned for differences in method. Only too often the technique used has not been stated; often the technique has been defined as that of some particular expert, but pursuit to that source reveals ambiguity of method. The amount of divergence between results by rival methods has seldom been analyzed. This paper contributes evidence from data on the human leg.

Material

The subjects were the same convicts described in the previous paper.

Landmarks

A desired dimension is obtained ordinarily either directly between two appropriate landmarks, or projectively by difference in level of the two landmarks above ground. The leg landmarks may therefore be listed, from above downward, showing for each its anthropometric name, symbol, and definition.

Iliospinale, is, the anterior superior iliac spine, at its lowest inmost tip, not its most prominent bulge.

Inguinion, in, midpoint of line from anterior superior spine to top of pubic symphysis. This point has been found in studies on cadavers to come extraordinarily close to the top of the head of the femur, and therefore is today the preferred proximal landmark (Jazuta, 1925, 1928; Davenport, 1933, 1935; Gjessing, 1934; Eickstedt, 1935).

Femurion, fe, the top of the head of femur; this point is already standard for measuring length on the clean bone, but the term is new. Since the living measurements under discussion in this paper are ordinarily intended to approximate bony dimensions, it follows that landmarks to be used on the living should advantageously approximate the end-points which have been standard for the clean bones. This principle will make evident our choices below of preferred measurements. Principally, we follow those who elect inguinion as best approximation to non-palpable femurion, to represent the top of the leg.

Trochanterion, tr, is the top of trochanter, not its most lateral prominence.

Symphysion, sy, is the top of symphysis pubis, not its anterior

bulge nor its lower edge, though each of these has been advocated by one observer.

Perinion, pe, is the top of the crotch with pressure (old landmark, new term).

Femorale, fe, is the most distal point on external femoral condyle.

Top of patella, bad landmark.

Tibiale, ti, the top of tibia internally, identically the standard osteological point for distal end of femur and proximal end of tibia, and therefore a preferred landmark.

Patellion, pa, new name for lower edge of patella, which is a bad landmark.

Sphyrion, sph, lowest tip of internal malleolus, corresponds to standard point for bony length of tibia. Therefore another preferred landmark.

Sphyrion fibulae, sph. f., tip of external malleolus, unsuitable.

Pternion, pte, back of the heel.

Akropodion, ap, front end of foot, usually the tip of big toe, occasionally of the second.

Leg-Length Averages and Variabilities

This paper deals only with measures of the extremity as a whole; the segments, thigh, shank, and foot will be treated in a separate paper. The averages are arranged in Table 1, beginning with the largest value. Beside each mean is shown its probable error, which permits us to calculate the probability that the differences between the white and negro means are significant. The criterion of significance used here is the ratio of the difference to its probable error, sometimes called the Critical Ratio, and is shown in the last column; a ratio of 3 or more is significant.

Iliospinal height, spinal height, iliospinale to ground. The actual distance from this point to the floor has been widely used in the literature, unfortunately with variable correction values; these have not always been specified by authors, and when specified have varied from 0 to 15, 20, 30, 34, 40, 50, 60 mm.; usually following Martin's table of 5 values for 5 stature-groups. The latter method was applied to obtain the values reported in this paper as "corrected," i.e., the item in the next paragraph.

Lower extremity. This dimension was derived for each individual by correction of gross iliospinal height.

TABLE 1
LEG-LENGTH CONSTANTS

Length		Race	Mean	±*pe*	*SD*	Critical Ratio *Diff./PE*
Iliospinal height	*is*	*W*	945	2.8	53.8	
		N	985	3.7	53.2	
		W — *N*	—40			9
Lower extremity	*is*, corr.	*W*	907	2.5	47.5	
		N	945	3.4	48.5	
		W — *N*	—38			9
Inguinion height	*in*	*W*	897	2.7	52.2	
		N	939	3.6	52.0	
		W — *N*	—42			9
Spine to malleolus	*is-sph*	*W*	883	2.7	50.6	
		N	926	3.6	52.4	
		W — *N*	—43			10
Symphysis height, lower measurment	*sy*	*W*	847	2.7	50.8	
		N	895	3.7	52.5	
		W — *N*	—48			11
Spine (corr.) to malleolus	*is-sph*, corr.	*W*	844	2.4	45.5	
		N	885	3.4	48.6	
		W — *N*	—41			10
Symphysis to Ext. malleolus	*sy-sph. f.*	*W*	807	2.6	49.7	
		N	858	3.6	50.9	
		W — *N*	—51			11
Rest-height	*S-Si*	*W*	789	2.5	48.2	
		N	834	3.6	50.7	
		W — *N*	—45			10

Inguinion height. The top of the femoral head, femurion on the bone, may be estimated in the living by correction of several landmarks. One, correction of spinal height, has just been shown as "lower extremity." In this section will be elaborated another, which we shall consider the preferred technique following the authors already referred to in defining inguinion in the section on landmarks. There the inguinion point was specified as half way between iliac spine and pubis. To obtain this value it may be convenient to proceed in one of three alternative ways:

(*a*) When only iliospinale has been recorded, we need to know a subtrahend which is half the difference in average levels for iliospinale and symphysis. We compute (Table *A*).

(*b*) When only pubic height has been recorded, we may estimate inguinion by using an addend. For the value of this addend we may use one of the subtrahends in the above text-table. By way of comparison, Topinard advocated 43 mm. as addend, which agrees well

TABLE *A*

	Mean stature in mm.	*is-sy* in mm.	Subtrahend In mm.	Subtrahend In % of *S*.
166 White convicts	1,693	98	49	2.89
97 Negro convicts	1,716	90	45	2.62
12 College athletes	1,774	84	42	2.37

with us; Mollison's 38 mm. and Martin's 35 mm. seem out of line.

(*c*) Much better than the two ways just given is the use of a prediction equation based on regression data. To obtain such data we need a series of subjects on each of whom both iliospinale and symphysis height have been recorded, the average of the two measurements on each man yielding inguinion for him. Then the regression of inguinion on iliospinale will yield a formula from which in future one can estimate an unmeasured inguinion from a measured iliospinale height, with more precision than by method (*a*) above. Similarly a regression of inguinion on symphysion will yield an equation more reliable for converting a measured pubic height to a preferred in-

TABLE 2
LEG REGRESSIONS

Y	*X*	Race	Regr. Coeff. *b*	*r*	s_E	s_b
Inguinion height	symphysis height	White	1.0113	.9831	9.565	.0146
		Negro	.9694	.9796	10.440	.0202
	iliospinale, uncorr.	White	.9553	.9828	9.649	.0139
		Negro	.9514	.9740	11.769	.0225
	iliosp.-sphyrion, uncorr.	White	1.0063	.9740	11.836	.0182
		Negro	.9643	.9733	11.924	.0231
	Rest-height, i.e., *S-Si*	White	1.0472	.9666	13.390	.0216
		Negro	.9858	.9615	14.276	.0286
Rest-height	symphysis height	White	.9127	.9612	13.302	.0203
		Negro	.9314	.9650	13.287	.0257
	sy-sphyrion fibulae	White	.9298	.9583	13.780	.0215
		Negro	.9483	.9527	15.398	.0307
	iliospinale height, corr.	White	.9635	.9480	15.348	.0251
		Negro	.9821	.9398	17.314	.0363
	iliosp.-sphyrion, corr.	White	.9915	.9347	17.140	.0293
		Negro	.9757	.9355	17.902	.0374
	acromion-stylion	White	1.4050	.8666	24.063	.0628
		Negro	1.3754	.8517	26.551	.0859
	relative sitting-height, *Si/S*	White	—30.502	—.8052	28.596	1.7437
		Negro	—25.661	—.7754	31.995	2.1217

guinion height, more accurately than by method (*b*) above. The necessary computations for all this are summarized in Table 2.

Spine to Internal Malleolus, is-sph. This dimension, which has some vogue among orthopedic surgeons, is not a very satisfactory dimension because any tilt of the pelvis tends to cause inaccuracy.

Symphysis height, sy. A dimension favored by some endocrinologists, but more inaccurate than most, as we have explained elsewhere.

Symphysis to External Malleolus, sy-sph. f. This dimension, approximately Pende's No. 4, is unusual and unsatisfactory, according to physical anthropologists.

Rest-height, S-Si. Though advocated by only a few students (Harris, 1924; Rössle and Böning, 1924; Schlesinger, 1925; Schlomka, 1927; Steggerda, 1928; Bean, 1933; Knott and Meredith, 1937), it has considerable appeal, and is treated here rather fully.

Conversion by Regression Equation

The notation has been detailed in a prior paper, so that here it will suffice to show as an example, the formula using the suitable values from Tables 1 and 2, to estimate inguinion height E for a white male adult in whom the observed symphysis height X was recorded as 995 millimeters:

$$\begin{aligned} E &= \overline{Y} + b(X - \overline{X}) \\ &= 897 + 1.0113\ (995 - 847) \\ &= 897 + 149.7 \\ &= 1{,}047 \text{ mm.} \end{aligned}$$

The necessary sampling errors will be: The standard error of estimate is $s_E = 9.56$; the standard error of the intercept or ordinate Y is $s_E/\sqrt{N} = 9.56/12.88 = 0.742$; the standard error of the slope or the regression coefficient is $s_b = 0.0146$.

Relative Leg-Length

The question may be asked, does leg-length remain a constant proportion of stature, alike in short and tall men? For evidence, take inguinion-height as the best leg-length, multiply by 100 and divide by stature, to obtain an index of relative leg-length, namely the inguinion/stature index. The values computed are (Table *B*).

Thus the negroes are longer legged than the whites in our series, as indeed has been judged in reports by others, though not on simultaneous observations for the two races.

TABLE *B*

	Mean	*SD*	*SE*
165 White convicts	52.9	1.37	0.107
97 Negroes	54.9	1.45	0.147

Proceeding now to the more detailed question posed above, all the individual indices were plotted; the distribution, while rather circular (therefore not published), shows a tendency of the index to increase with the tallness of the subject, the correlation coefficient being 0.45 for the whites, 0.37 for the negroes. The regressions of the index on stature are:

165 whites $Y = 38.1 + 0.00878\,X$
97 negroes $Y = 41.5 + 0.00780\,X$

Direct vs. Compound Measurements

A given dimension is sometimes measured directly, but frequently obtained by compounding certain sums (or differences). The latter method is convenient, but obviously tends to introduce errors in proportion to the number of items used in obtaining the compound value. For a single subject, Todd (1925) wrote:

> Computation of a dimension by the summation of factorial measurements is reliable only to within about 5 mm. if two factors are employed, and to within about 10 mm. if three factors are used. . . . Note that these figures are average deviations, not deviations of one average from another. The latter are naturally smaller.

The errors may be indicated quantitatively by finding the variability larger for compound value than for direct value. In other words, when an identical dimension is measured by two or more methods, that method is preferable which is associated with the smallest standard deviation. The values by the two methods, for several leg dimensions, have been computed and show the errors to be considerable for our 23 tall individuals. For the sake of conciseness, and since most often studies generalize, not from individuals, but from averages, our computations are condensed in Table 3 and Table 4.

A dimension as a compound of two is seen to be in error, alike in Todd's instances and ours, by about 0.4 per cent of the direct, except for the difficultly measurable foot-height when the error amounts to about 3 per cent.

TABLE 3
DIRECT VS. COMPOUND MEASUREMENTS

Dimension	Method	Mean	*SD*	*SE* of Mean
Iliospinal height	(*is-ti*) + (*ti*-ground)	1,105	30.7	6.41
	Direct	1,103	31.1	6.48
	(*is-ti*) + (*ti-sph*) + (*sph-gr*)	1,103	31.9	6.66
	(*is-sph*) + (*sph-gr*)	1,101	32.0	6.67
Inguinion height	Direct	1,045	27.8	6.07
	(*is* + *sy*)/2	1,055	29.8	6.50
Iliospinale-int. mall.	(*is-ti*) + (*ti-sph*)	1,019	28.7	5.99
	Direct	1,015	30.4	6.35
Tibia	Direct	440	12.8	2.74
	(*ti-gr*) — (*sph-gr*)	442	14.4	3.07
Foot-height	(*ti-gr*) — (*ti-sph*)	85.7	6.66	1.42
	Direct	83.8	7.28	1.55
	(*is-gr*) — (*is-sph*)	87.4	8.39	1.79

TABLE 4
ERROR OF COMPOUNDED DIMENSIONS

		Deviation in % of direct vs. compound of 2 items	vs. compound of 3 items
Todd arm length	*a*	—0.45	
	b		+1.39
	c	+0.48	
Iliospinal height	*a*	+0.18	
	b		0
	c	—0.18	
Inguinion height		+0.96	
is-sph		+0.38	
Tibia		+0.45	
Sphyrion height	*a*	+2.27	
	b	+4.30	

SUMMARY

Regression equations are given by which the leg-length actually measured in various ways can be converted to the value probable if a preferred method had been used.

The methods preferred in this paper have been either inguinion-height (the average of the heights of anterior iliac spine and of the pubes), or the so-called rest-height (stature minus sitting-height, a measurement indefensible in theory but convenient in practice).

The correlation between the length of the leg and the length of the arm (from acromion to wrist, i.e., without hand) is high, $r = 0.87$ in white adult males, 0.85 in negroes. This was computed because, although high correlation between the extremities is often assumed, it has been doubted.

In orthopedic operations which involve inequality in length of the lower extremities, some of the remarks in this paper may find practical use.

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SEGMENT-PROPORTIONS IN THE EXTREMITIES, WITH SPECIAL REFERENCE TO TALL MEN AND GIANTS*

A. L. KASLOW AND H. GRAY

The Men Students' Health Service, Stanford University, and the Stanford University School of Medicine, San Francisco, California
(Received for publication, August 25, 1941)

The relative proportions of the three segments composing each limb, as distinct from the proportion between limb-length and body-length, have been little studied, and are worth study, (*a*) for pure science, (*b*) for possible use in estimating fitness for particular sports, (*c*) for diagnosis of abnormalities of growth, such as dwarfism or giantism.

ARM SEGMENTS

The upper extremity may be first considered, split into three fractions each reckoned as a percentage of the whole limb. The proximal segment is the humerus, always measured from tip of acromion to upper edge of the radial head (radiale). The mid-segment is the radius, measured from radiale to tip of radial styloid (stylion). The end-segment is the hand, measured either (*a*) from stylion to tip of midfinger (dactylion), or (*b*) from dactylion to the wrist-point carpale, *ca*, which is the middle of the diagonal line from tip of radial styloid to tip of ulnar styloid; the latter yields hand-lengths longer by three millimeters than the former, as shown by Bayer and Gray (1933), who preferred the carpale landmark in agreement with various physical anthropologists; for the present purpose the distinction is sufficiently slight to be negligible.

Source data suitable for the desired analysis have been traced for several categories of people, some supplementary college men have been measured by us, and the desired fractions computed.

Usual Proportions within the Arm

For males two independent sources yield extraordinarily like values: Upper-arm 42 per cent, fore-arm 33 per cent, hand-length 25 per cent of total arm (see Tables 1 and 2).

*Aided by the Rockefeller Fluid Research Fund.

TABLE 1

ARM SEGMENTS

In boys from 6 to 20 years, calculated from data of Schwerz.

Age	*N*	Stature	Relative in % of total			
			Upper arm	Forearm	Hand	Total
6- 7	46	1,146	40.9	33.8	25.3	100.0
7- 8	97	1,169	41.2	33.4	25.4	100.0
8- 9	84	1,211	41.8	33.3	24.9	100.0
9-10	80	1,282	41.8	33.6	24.6	100.0
10-11	67	1,323	41.9	33.9	24.2	100.0
11-12	81	1,359	42.0	33.8	24.2	100.0
12-13	76	1,398	41.9	33.4	24.7	100.0
13-14	107	1,451	41.9	33.5	24.6	100.0
14-15	86	1,515	41.8	33.7	24.5	100.0
15-16	62	1,540	41.9	33.8	24.3	100.0
16-17	36	1,571	42.3	33.5	24.2	100.0
17-18	31	1,615	42.1	33.0	24.9	100.0
18-19	27	1,641	42.1	33.0	24.9	100.0
19-20	29	1,662	42.4	33.2	24.4	100.0
Over 20	51	1,694	42.4	33.1	24.5	100.0
Pool of two shortest (youngest) groups $N = 143$		1,162	41.0	33.6	25.4	100.0
Pool of similar central groups, 8 such groups $N = 643$		1,385	41.9	33.6	24.5	100.0
Pool of five older groups $N = 174$		1,641	42.3	33.1	24.6	100.0
Pool of all 15 groups $N = 960$		1,398	41.9	33.5	24.6	100.0

Sex

Women show a different division of the segments: surprisingly the hand constitutes a larger fraction of the arm than in men, while both forearm and humerus account for lesser fractions of the arm than in men. The relationships are true in both whites and negroes (see Table 3).

Race

Negro adults differ from whites. Contrary to common notions, the negro hand is a smaller part of arm-length than in whites. The forearm composes a larger fraction, and the humerus a smaller fraction than in whites. These relations are true in both males and in females (see Table 3).

Chinese males, from 2 to 20 years, when compared with both white and negro races, show the hand-fraction larger, the radius about the same, the humerus shorter (see Table 4).

TABLE 2

ARM SEGMENTS

In adult males from 20 to 34 years, calculated from data of Bach.

		Relative in % of total			
N	*S*	Upper arm	Forearm	Hand	Total
3	147	43.3	32.0	24.7	100.0
7	150	42.0	33.3	24.7	100.0
25	153	42.1	32.6	25.3	100.0
56	156	42.9	32.4	24.7	100.0
139	159	42.8	32.2	25.0	100.0
240	162	42.7	32.5	24.8	100.0
307	165	42.7	32.4	24.9	100.0
265	168	42.8	32.5	24.7	100.0
228	171	43.0	32.4	24.6	100.0
155	174	42.9	32.4	24.7	100.0
106	177	43.0	32.4	24.6	100.0
40	180	43.1	32.4	24.5	100.0
13	183	42.7	32.9	24.4	100.0
13	186	42.9	32.7	24.4	100.0
4	189	43.1	33.1	23.8	100.0
2	192	43.7	32.5	23.8	100.0
Pool of three shortest groups $N = 35$	152	42.5	32.6	24.9	100.0
Pool of central groups with similar percentages, 9 such groups $N = 1,536$	167	42.9	32.4	24.7	100.0
Pool of four taller groups $N = 32$	186	43.1	32.8	24.1	100.0
Pool of all sixteen groups $N = 1,603$	167	42.9	32.5	24.6	100.0

TABLE 3

ARM SEGMENTS BY SEX AND RACE

Calculated by weighting values from various sources.

				Fraction of total arm		
Sex	Race	*N*	Stature	Humerus	Radius	Hand
Male	White	2047	1,674	42.8	32.5	24.7
	Negro	100	1,744	42.1	33.3	24.6
Female	White	504	1,592	42.0	32.3	25.7
	Negro	132	1,622	41.4	33.0	25.6

Stature

In order to determine the effect of increasing tallness on the fractions of the arm, we show the results on 23 tall athletes at Stanford

TABLE 4

ARM SEGMENTS

In Chinese boys from 6 to 20 years, calculated from data of Appleton.

Age	*N*	Relative in % of total				
		Stature	Upper arm	Forearm	Hand	Total
6	23	1,112	40.9	31.7	27.4	100.0
7	22	1,156	40.3	32.8	26.9	100.0
8	42	1,206	40.6	32.4	27.0	100.0
9	34	1,246	40.7	32.1	27.2	100.0
10	53	1,287	40.6	32.5	26.9	100.0
11	33	1,341	40.8	32.6	26.6	100.0
12	39	1,391	40.8	32.5	26.7	100.0
13	32	1,451	41.6	32.0	26.4	100.0
14	21	1,538	41.0	32.4	26.6	100.0
15	28	1,597	41.1	32.6	26.3	100.0
16	22	1,621	41.1	32.6	26.3	100.0
17	10	1,650	41.5	33.1	25.4	100.0
18	11	1,631	41.8	32.8	25.4	100.0
19	7	1,686	41.2	32.6	26.2	100.0
20	11	1,654	41.2	33.2	25.6	100.0
Pool of ages 6 and 7	45	1,114	40.6	32.2	27.2	100.0
Pool of ages 8 to 15	282	1,359	40.9	32.4	26.7	100.0
Pool of ages 16 to 20	61	1,572	41.3	32.9	25.8	100.0
Pool of all 15 groups	338	1,364	41.0	32.5	26.5	100.0

(Table 5), a summary of the preceding tables together with averages derived from some additional authors (Table 6), a condensed survey (Table 7), and a diagram (Figure 1).

Examination of these in order of increasing height shows that the hand-fraction also increases, but by a surprisingly small amount, even in the giants. The main feature is that the forearm-fraction increases as stature increases; the humerus therefore necessarily shows a decreasing fraction of the arm. These inferences are plainest in Figure 1, from the open circles representing three male groups of markedly different heights. The precise values for the males are in Table 7, and there also are values for two contrasted groups of women. It is disappointing, when we plot the values from each of the categories from Table 6, as solid spots, to observe the enormous variability of these human averages. Variation is even worse among the individuals whom we have personally measured, and on the giants collected from the literature, hence these have not been plotted.

TABLE 5

ARM SEGMENTS

Calculated from our data on 23 tall athletes at Stanford.

Subject	*S*	Relative in % of total			
		Upper arm	Forearm	Hand	Total
J. K.	1,875	42.2	33.7	24.1	100.0
W. A. R.	1,881	41.1	33.0	25.9	100.0
P. N.	1,881	42.1	32.9	25.0	100.0
R. R. P.	1,885	42.1	32.6	25.3	100.0
L. R.	1,885	42.7	33.8	23.5	100.0
J. C. W.	1,886	42.4	31.7	25.9	100.0
W. C.	1,892	41.9	33.1	25.0	100.0
S. L. A.	1,894	41.0	33.7	25.3	100.0
R. De L.	1,895	41.3	33.3	25.4	100.0
J. C. T.	1,896	42.6	33.4	24.0	100.0
J. R. M.	1,897	41.6	33.2	25.2	100.0
A. J. F.	1,901	40.8	33.7	25.5	100.0
J. N. S.	1,901	42.9	32.5	24.6	100.0
W. R. M.	1,902	41.5	32.8	25.7	100.0
R. W. B.	1,906	41.4	33.5	25.1	100.0
E. W. H.	1,921	42.3	33.5	24.2	100.0
L. B. B.	1,922	43.1	32.8	24.1	100.0
W. G. A.	1,927	41.2	33.4	25.4	100.0
M. H.	1,930	42.8	33.3	23.9	100.0
P. C. A.	1,947	41.3	34.1	24.6	100.0
R. M. P.	1,963	42.6	33.0	24.4	100.0
G. S. B.	1,971	41.5	32.6	25.9	100.0
R. K. T.	2,030	43.6	31.7	24.7	100.0
Means	1,913	42.0	33.1	24.9	100.0
SD	36	0.76	0.61	0.71	
SE of Mean	8	0.16	0.13	0.15	

LEG SEGMENTS

As before, the extremity may be split into three parts and each reckoned as a fraction of the total. The near-segment is the femur, for which the preferred landmarks are inguinion to tibiale (Gray, to be published); and the mid-segment is the tibia, i.e., tibiale to internal malleolus (sphyrion).

The distal segment is less obvious; indeed, since the tibia is not attached to the end of the heel, and since it is attached at an angle, attempts at a total have been supposed to be unreasonable. We submit three alternatives and a solution. The three distal alternatives may be:

(*a*) Internal malleolus to the ground, so-called sphyrion height, or foot-height.

(*b*) Internal malleolus (*sph*) to tip of toes (*ap*), when the foot

TABLE 6
ARM SEGMENTS BY STATURE

Source	*N*	Category	Stature	Segment in per cent of whole arm: Upper arm	Fore-arm	Hand
Appleton, '25	73	Chinese girls aged 16-20	1,542	41.0	32.4	26.6
Steggerda '40	100	Navajo Indian girls aged 19 years	1,556	41.2	33.0	25.8
Appleton '27	61	Chinese boys 16-20 years	1,572	41.3	32.9	25.8
Schmidt '29	100	Schizophrenic women	1,576	42.8	32.0	25.2
Todd & Lindala '28	32	Negro women	1,586	42.1	33.0	24.9
Todd & Lindala '28	36	White women	1,597	43.2	32.2	24.6
Grützner '27	47	Swiss girls age 18	1,614	43.6	32.8	23.6
Steggerda '32	60	Dutch women	1,618	41.3	31.3	27.4
Steggerda, *et al.*, '29	100	College girls, white	1,628	41.6	31.8	26.6
Steggerda '40	100	Negro college girls	1,633	41.2	33.0	25.8
Schwerz '10	174	Boys 16-20 years	1,641	42.3	33.1	24.6
Collected from the literature and calculated by us	5	Giant and acromegalic females	1,644	40.4	33.8	25.8
Bach '26	1,603	White males	1,672	42.9	32.5	24.6
Schmidt '29	100	Schizophrenic males	1,696	42.4	32.8	24.8
Todd & Lindala '28	100	White males	1,706	43.3	32.4	24.3
Steggerda '32	70	Dutch males	1,732	42.3	30.0	27.7
Todd & Lindala '28	100	Negro males	1,744	42.1	33.3	24.6
Kaslow & Gray	23	Tall Stanford athletes	1,913	42.0	33.1	24.9
Collected from the literature and analyzed by us		Giant and acromegalic males	2,037	41.6	33.4	25.0

TABLE 7
ARM SEGMENTS BY STATURE

		N	Stature	Per cent of total arm: Upper arm	Fore-arm	Hand
Male white	Average of several groups of normals	2,047	1,674	42.8	32.5	24.7
	Tall athletes	23	1,913	42.0	33.1	24.9
	Giants and acromegalics	29	1,915	41.6	33.4	25.0
Female white	Average of several normal groups	504	1,592	42.0	32.3	25.7
	Giants and acromegalics	5	1,644	40.4	33.8	25.8

is extended as far as possible to lie in line with the shin, analogous to hand-length measured in line with the arm.

(*c*) Heel to toe, technically pternion-akropodion (*pte-ap*), the ordinary foot-length.

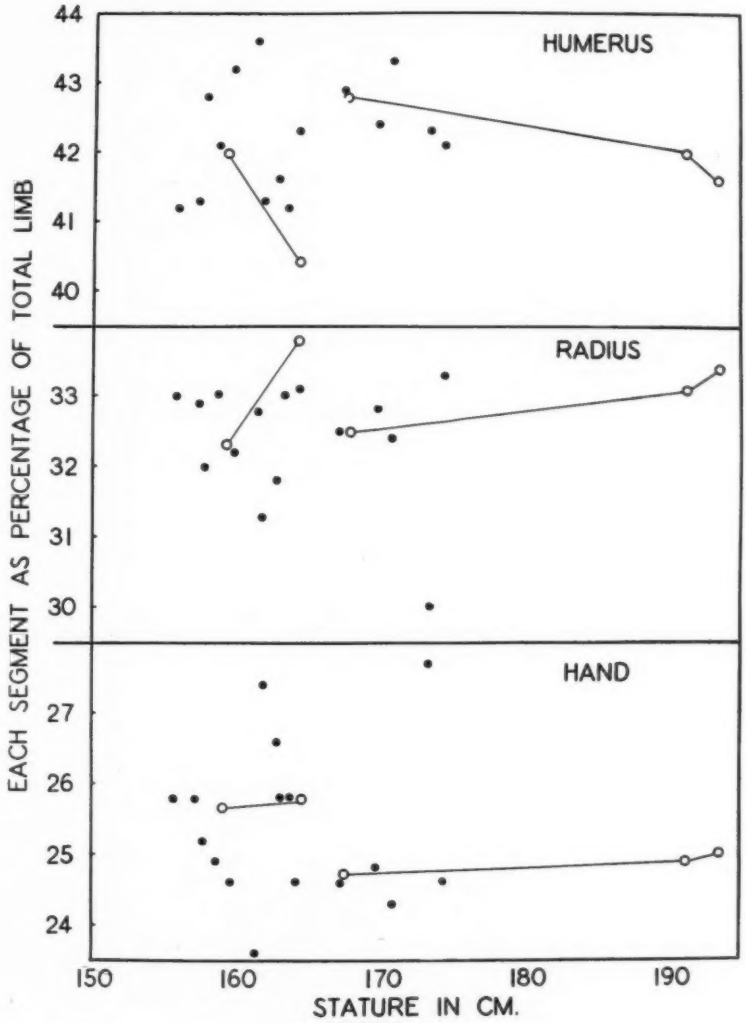
HUMERUS
RADIUS
HAND
EACH SEGMENT AS PERCENTAGE OF TOTAL LIMB
44
43
42
41
40
33
32
31
30
27
26
25
24
150
160
170
180
190
STATURE IN CM.

FIGURE 1

Using each of these in turn we obtain three corresponding total leg lengths, which may be termed:

Leg Direct = Femur and Tibia + (*a*) foot-height,
Leg Extended = Femur and Tibia + (*b*) malleolus to toe,
Leg Cumulated = Femur and Tibia + (*c*) foot-length.

The "leg direct" is identical with the dimension called inguinion-height, which has been urged elsewhere as preferable when comparing the leg with other parts of the body. But for segmental study within the leg, which is our present problem, we shall now show that the "leg-cumulated" is preferable.

Suitable data for the necessary analysis have not been found, and have therefore been measured by one of us (A.L.K.) on a dozen normal adult students in the Men's Health Service at Stanford University. On each subject the observations comprised the thigh, shank, and the three alternative end-segments (*a*), (*b*), (*c*). For each subject, the three corresponding alternatives for total leg were computed, together with the segmental proportions under each such alternative. The dozen subjects were then averaged. When distal item (*a*) was used, it was found to constitute 7.6 per cent of the "direct" leg; when distal item (*b*) was tried, it was found to constitute 19.0 per cent of the "extended" leg; and when item (*c*) was applied, it constituted 23.1 per cent of the "cumulated leg." Since this last named fraction is by far the closest to the fraction 25 per cent already reported for the distal segment in the upper extremity, we conclude that the best total leg-length for the present purpose is the third alternative, namely the leg cumulated by adding thigh-length (inguinion to tibiale), plus shank (tibiale to internal malleolus), plus foot-length (heel to toe).

Pursuant to this conclusion we display the results of our calculations on such series as we could find. It is clear that segmental proportions in the leg agree remarkably with those already shown for the arm.

Sex

In women the foot comprises a smaller fraction of the leg than in males; this is as expected; let us recall again the surprising finding for the upper extremity, that the female hand formed a larger fraction than in men. The middle segment of the leg in women was longer than in men, again reversing the relationship noted for the arm. The proximal fraction of the leg in women composed a smaller part of the whole than in men; this relationship is the same as found for

the upper limb. The female-male relationships described were true in both whites and negroes (see Table 8).

TABLE 8

LEG SEGMENTS BY SEX AND RACE

Sex	Race	*N*	Stature	Fraction of total leg		
				Thigh	Shank	Foot
Male	Whites	400	1,682	43.0	33.6	23.4
	Negroes	100	1,744	42.2	35.3	22.5
Female	Whites	412	1,592	42.7	34.0	23.3
	Negroes	32	1,586	42.1	35.4	22.5

Race

Contrary to prevalent notions, just as was found for the arm, the negro foot forms a smaller proportion of the leg than in whites. The mid-segment forms a larger fraction of the cumulated leg, and the near-segment a smaller part, than in whites. These race relationships are true alike for both sexes (see Table 8).

Leg-Segments and Stature

As found for the arm, increasing stature has little effect on the distal leg-segment, increases the mid-segment, and decreases the proximal segment. Condensing our computations, we show seven general categories in Table 9 and Figure 2. The leg shows, better than the

TABLE 9

LEG SEGMENTS AND STATURE

Categories	Sex and No.	Stature	Percentage of total leg		
			Thigh	Shank	Foot
Manics and schizophrenics	187 *F*	1,574	43.4	33.5	23.1
Normal Swiss girls	225 *F*	1,607	42.1	34.5	23.4
Manics and schizophrenics	300 *M*	1,677	43.1	33.3	23.6
Cleveland hospital patients	100 *M*	1,706	42.7	34.7	22.6
Stanford normals	12 *M*	1,776	42.5	34.4	23.1
Stanford tall athletes	23 *M*	1,913	41.7	35.3	23.0
Giants and acromegalics	30 *M*	1,968*	40.8	36.1	21.9

*The average stature for the giant and acromegalic class differs from the stature given in the analogous table for arm-length, because the individuals in the two groups are not identical; some of the reported cases in the literature had suitable measurements for the arm, some for the leg, and some (22 to be exact) for both.

arm, the progressive changes in segmental proportions with increasing stature.

For our 23 tall athletes the details are recorded in Table 10.

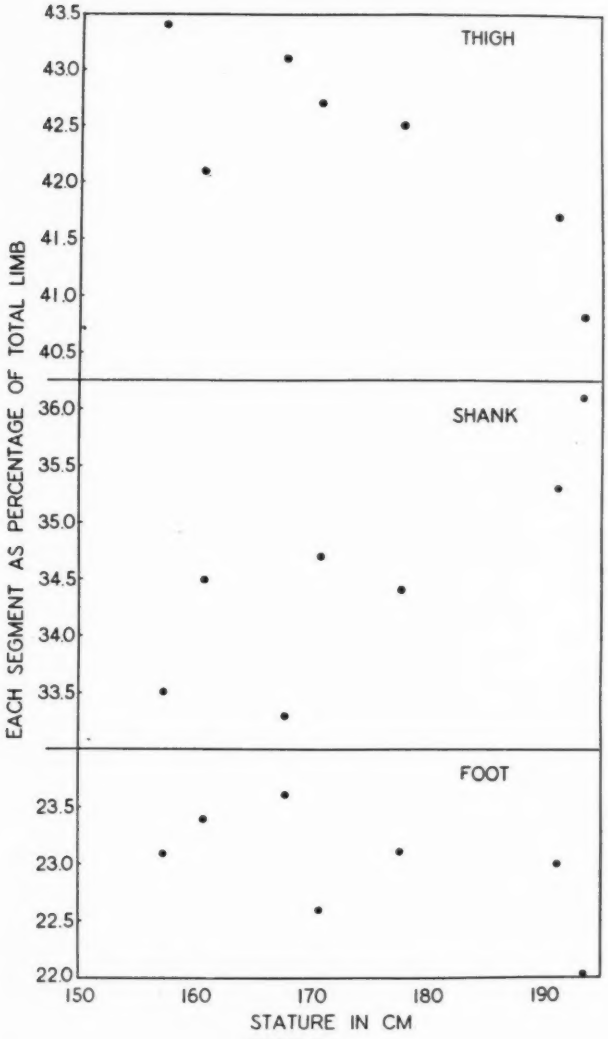
THIGH
SHANK
FOOT
EACH SEGMENT AS PERCENTAGE OF TOTAL LIMB
43.5
43.0
42.5
42.0
41.5
41.0
40.5
36.0
35.5
35.0
34.5
34.0
33.5
23.5
23.0
22.5
22.0
150
160
170
180
190
STATURE IN CM

FIGURE 2

TABLE 10

LEG SEGMENTS IN TALL ATHLETES

Calculated from our data on 23 tall athletes at Stanford, all 6 feet 1 inch or more.

Subject	*S*	Relative to total leg		
		Thigh	Shank	Foot
J. K.	1,875	42.6	34.8	22.6
W. A. R.	1,881	42.4	34.6	23.0
P. N.	1,881	40.5	37.5	22.0
R. R. P.	1,885	42.0	34.9	23.1
L. R.	1,885	41.1	36.4	22.5
J. C. W.	1,886	40.7	36.4	22.9
W. C. A.	1,892	42.5	34.4	23.1
S. L. A.	1,894	42.0	35.1	22.9
R. De L.	1,895	41.4	36.1	22.5
J. C. T.	1,896	41.2	35.7	23.1
J. R. M.	1,897	40.5	37.1	22.4
A. J. F.	1,901	41.9	35.6	22.5
J. N. S.	1,901	42.2	34.6	23.2
W. R. M.	1,902	40.5	36.3	23.2
R. W. B.	1,906	41.2	35.9	22.9
E. W. H.	1,921	41.7	34.1	24.2
L. H. B.	1,922	41.9	35.5	22.6
W. G. A.	1,927	41.1	34.7	24.2
M. H.	1,930	42.6	34.7	22.7
P. C. A.	1,947	42.3	34.3	23.4
R. M. P.	1,963	43.6	34.5	21.9
G. S. B.	1,971	41.5	35.2	23.3
R. K. T.	2,030	42.6	33.6	23.8
Mean	1,913	41.7	35.3	23.0
SD	36	.82	.99	.59
SE of Mean	8	.17	.21	.12

The giants' measurements were culled from the literature; those which had been observed from landmarks (particularly iliospinale, trochanterion, symphysion) other than those preferred for the present paper, were translated by means of the conversion equivalents reported by us elsewhere, and finally the fractions computed. The individual fractions are not reproduced, but the averages are listed in Table 9.

FOOT HEIGHT

For segmental indices this dimension has above been shown to be inferior to foot-length. Nevertheless, a method for estimating its magnitude is necessary, in order to correct leg-lengths as frequently measured to the floor, in order to obtain net shank-length, the mid-segment used for calculating segmental proportions.

There are two measures of foot-height: internal malleolus (sphyrion) and external malleolus (sphyrion fibulae). The latter is not only more difficult to measure, but also is theoretically inferior, as

remarked previously. Either will at times be needed as a correction and therefore we put on record the results of our calculations on male adults from such sources as we have been able to find (see Tables 11 and 12).

TABLE 11

RELATIVE INTERNAL MALLEOLUS HEIGHT = 100 SPHYRION/STATURE

Category	Source	*N*	Stature	Mean	*SD*	*SE* of Mean
Boys—Age 9-18	Davenport	105 *M*	1,325-1,732	3.87		
French Boys—Age 13-17	Godin	100 *M*	1,452-1,636	4.49		
Schizophrenics	Schmidt	100 *F*	1,576	4.19		
Negro	Todd and Lindala	32 *F*	1,586	4.03		
White	Todd and Lindala	36 *F*	1,597	3.94		
Swiss girls—Age 18	Grützner	47 F	1,614	4.44		
Smith College girls	Steggerda, *et al.*, '29	100 *F*	1,628	4.76		
Schizophrenics	Plattner	100 *M*	1,642	4.02		
White convicts		165 *M*	1,693	3.68	0.332	0.0258
Schizophrenics	Schmidt	100 *M*	1,696	4.36		
Aborigines	Burston	61 *M*	1,699	4.18	0.331	0.0424
White	Todd and Lindala, '28	100 M	1,706	4.29		
Negro convicts		97 M	1,716	3.47	0.307	0.0312
Negro college students	Todd and Lindala, '28	100 *M*	1,744	3.97		
		12 *M*	1,776	4.13	0.465	0.1342
Tall athletes		23 M	1,913	4.40	0.372	0.0776

TABLE 12

RELATIVE EXTERNAL MALLEOLUS HEIGHT = 100 SPHYRION FIBULAE/STATURE

Category	*N*	Stature	Mean	*SD*	*SE* of Mean
White convicts	165	1,693	2.34	0.296	0.0230
Negro convicts	97	1,716	2.20	0.400	0.0406
White students	12	1,776	3.26	0.333	0.0960

The units of measure to be tabulated might be taken either absolutely in millimeters or relatively. The latter proportional expression will be much more convenient to use, provided the proportion (of foot-length to say stature) remains constant with increasing stature; in fact a plot (not reproduced) of 166 white male convicts whose heights ranged from 152 to 187 cm., shows this assumption of constancy to be justified.

Summary

Peculiarities of growth in the extremities, as analyzed into the proportions of the three segments (near, mid, and distal) within the limb, have been merely touched on in the literature, and seem worth more evidence, which is therefore presented.

Women show the hand-fraction larger than in men, contrary to anticipation; the foot-fraction smaller, as we should expect.

Negroes show both hand and foot fractions smaller than in whites, contrary to expectation. The Chinese race on the other hand have a hand fraction larger than in whites.

The taller the stature of an individual, the greater the percentage of the limb represented by the proximal segment, the lesser the percentage represented by the mid-segment. The distal segment remains a relatively constant fraction of the total extremity.

We are indebted to Dr. Charles E. Shepard, director of the Men Students' Health Service, for facilitating the observations; to Dr. Bryce Boyer and Mrs. James H. Miller for assistance with the calculations.

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ANIMAL GROWTH AND NUTRITION, WITH SPECIAL REFERENCE TO THE RAT

LOIS ZUCKER, LILIAN HALL, MARGARET YOUNG, AND THEODORE F. ZUCKER

Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City
(Received for publication, October 17, 1941)

NUTRITIONAL REQUIREMENTS AND LAWS OF GROWTH

Probably a majority of the formulations of growth for rats have been based upon or fitted to the data of Donaldson (Donaldson, *et al.*, 1906), whose weight time curves were obtained from rats on a diet poorly characterized and certainly deficient in a number of factors. If a growth law were to be worked out for rats on deficient diets, account would have to be taken of the deficiencies in the sense that perhaps for each of the 30 odd nutritional essentials the formula would need a parameter giving expression to the fraction of the total requirement which is present, together with a provision for varying requirements at varying ages. The formulation suggested by Wetzel (1937) and not yet applied to rats, embodies such general principles, but provides only five parameters in the expression for food intake. Teissier (1936) considered only quantity of food as a variable, whereas quality suffers far more frequently and in a great diversity of ways. Robertson (1923) was quite aware of the importance of quality and discussed the various nutritional requirements as they were known in 1923 in some detail. Nevertheless his growth equation contains one parameter proportional to the "nutritional level." However it is arrived at, a single parameter covering differences in quality of diet is equivalent to the assumption that the organism has only one series of responses to all the different dietary deficiencies which can affect growth, corresponding to the various values of this overall parameter. We cannot expect a given degree of Vitamin *A* deficiency to be equivalent to a given degree of, let us say, phosphorus deficiency in their effects on growth. We cannot expect growth on deficient diets simply to reproduce normal growth on a smaller scale.

An alternate approach to the problem is to restrict consideration to data obtained on diets supplying every nutritional requirement in full at all ages, so that diet can be ignored in the formulation. The organism has a definite scheme of growth which can be hindered by nutritional deficiencies but which cannot be indefinitely speeded up beyond certain inherent limitations by nutritional means. If further dietary changes of any kind result in no further increase in growth rate at any age, then all nutritional requirements for growth must have been satisfied and the inherent growth determinants, whatever their nature, are free to fix the shape of the growth curve. This of course has been the assumption in growth equations which do not have separate parameters concerned with nutritional effects. But it cannot be too frequently emphasized that the older growth data failed to meet this requirement.

Nutritional Aspects of Pre-Weaning and Post-Weaning Growth

It is probable that post-weaning growth curves of rats fed *ad libitum* on current good diets are at least close approaches to the desired nutritionally uninhibited growth. During earlier life, however, the food intake is not entirely determined by the needs of the individual as an independent organism. The possibility of a restricted food supply exists, and there is evidence that this is the case in the negative correlations which have been established in mice and rats between litter size and the individual's birth weight and growth rate after birth (King, 1915*a;* Bluhm, 1929; Kopec, 1932; Crozier and Enzmann, 1935-36; Crozier, 1939), and also in the improved growth rate of suckling mice when more milk is made available (MacDowell, *et al.,* 1930). Unfortunately most of this work was done without due consideration of the quality of the diet offered the mothers. Naturally if one does not supply the mother with enough of a given essential food factor for her requirements and those of the young, and if there is not a sufficient store of this factor in the body of the mother, either quantity or quality of milk, or both, suffer, and a nutritional growth inhibition sets in which is probably complex in nature and is certainly arbitrary. Reference can be made to Sure (bibliography in Sure, 1940) for effects on milk quality, to Daggs (1931, 1935, 1938), and to the chapter on lactation in Maynard's book (1937) for effects on milk quantity and length of lactation period.

An optimal diet for the mother is also therefore an essential con-

sideration for the study of pre-weaning growth. However, even with optimal diets it cannot be concluded necessarily that the mother will supply the nurslings with as much milk as they can take if the litter is very large. There is a question whether the control of appetite by bodily requirements has as yet been established in these very young rats. In any case the nutritional differences before and after weaning suggest the advisability of independent treatment of pre- and post-weaning growth data (compare MacDowell, *et al.*, 1930). More cogent reasons for this division are given in the succeeding paper.

Experimental

Diet

See Zucker, *et al.* (1941).

Colony Management

Albino rats of the Osborne-Mendel-Sherman strain were used. There is only random inbreeding in the colony. Litters range in size from 2 to 11; large litters are reduced immediately after birth, and small litters are enlarged wherever possible by fostering rats discarded from large litters born the same day. The mean adjusted litter size of the rats of Table 1 is 6.2; the mean size has fallen to 5.5 at weaning. The young begin to eat stock diet spontaneously at about 2½ weeks, and weaning is completed on the 28th day by separating the young from the mother. At this time the sexes are separated and the animals to be used for breeders are selected. In selecting breeding stock we try to discard sickly inferior animals and runts. There is a tendency to choose rats which are somewhat above the average in weight at this age. This is partly a criterion of health, vigor, and nutritional history, but is partly determined by inherent size difference which is maintained throughout life. Inherent size varies largely in the colony as shown in Figure 1. Increase in average size of the rats of the colony has not resulted from this practice to date, although it is possible to breed rats for increased size by intensive and consistent selection. Animals which are sick or lose weight consistently, males which are sterile (by test with a female in oestrous), and females which do not show regular oestrous cycles or which produce poor quality young are discarded.

TABLE 1

GROWTH OF FEMALE BREEDERS IN 1936 AND 1937

Age range Days	*N*	Av. wt. grams	σ
121-125	11	201	15.1
126-130	13	196	11.5
131-135	16	200	10.5
136-140	30	201	14.9
141-145	37	192	15.6
146-150	26	199	12.0
151-155	26	196	12.4
156-160	22	200	12.7
161-165	29	201	11.0
166-170	41	199	16.8
171-175	28	198	18.7
176-180	19	202	15.1
181-185	18	204	15.0
186-190	12	209	16.1
191-195	13	210	11.3
196-200	14	221	18.0
201-205	26	218	13.2
206-210	23	214	17.4
211-215	13	213	20.6
216-220	26	216	15.1
221-225	22	214	14.5
226-230	25	209	18.3
231-235	26	215	18.8
236-240	16	212	19.0
241-245	19	211	14.3
246-250	27	223	13.5
251-255	14	228	17.9
256-260	17	229	22.8
261-265	19	226	19.6
266-270	14	222	22.9
271-275	13	229	10.7
276-280	19	225	15.0
281-285	15	227	17.1
286-290	15	226	19.4
291-295	15	222	13.2
296-300	22	227	14.2
301-305	17	231	17.3
306-310	15	233	21.4
311-315	13	232	22.8
316-320	17	232	24.8
321-325	9	219	25.3
326-330	7	245	17.4
331-335	8	240	16.5
336-340	16	236	20.6
341-345	11	231	14.6
346-350	17	229	19.7
351-355	13	224	15.2
356-360	7	251	25.4
361-365	14	238	15.4
366-370	10	241	13.3
371-375	11	229	12.6
376-380	11	240	19.1
381-390	10	233	22.4
391-400	18	242	20.8
401-410	10	248	14.3
411-420	14	243	21.1
421-430	9	248	26.4
431-440	8	246	14.3
441-460	15	247	20.1
461-480	14	246	25.1

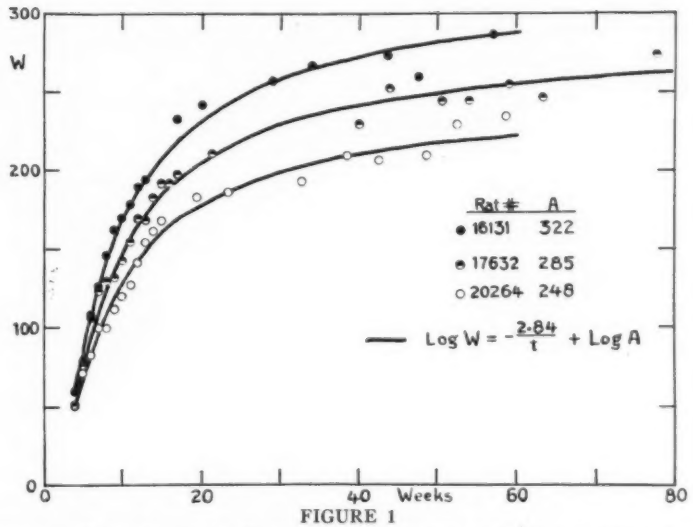

FIGURE 1

GROWTH OF THREE INDIVIDUAL FEMALES OF OUR COLONY OF DIFFERENT INHERENT SIZES
The equation fitted to the data, and also to the three data curves of Figure 2 with suitable adjustment of the size factor *A*, was first described by Zucker, *et al.* (1941). It will be discussed in detail in a paper to follow.

Post-Weaning Data

The 311 females and 42 males comprising the breeding stock in 1936 and 1937 were each weighed at weekly intervals from 4 to 17 weeks of age. The data have already been reported (Zucker, *et al.*, 1941). The subsequent growth of the same females weighed at irregular intervals between pregnancies is recorded in Table 1. Each animal contributes an average of 4.6 weights. Individuals were bred from one to eight times. Among these rats are the three individuals whose growth is shown in Figure 1. Unfortunately the first groups in Table 1, the rats which finished their first pregnancy youngest, represent a selected group of large inherent size, because some large females were bred younger than small ones. This explains the apparent bump in the curve where the data of Table 1 meet the earlier 14-17-week data (see Figure 2). Inherently small rats are concentrated a little further along for the same reason. This fact imposes a cyclic character upon the course of the mean weights which is not characteristic of the individual curves. We are unable to confirm for our rats the

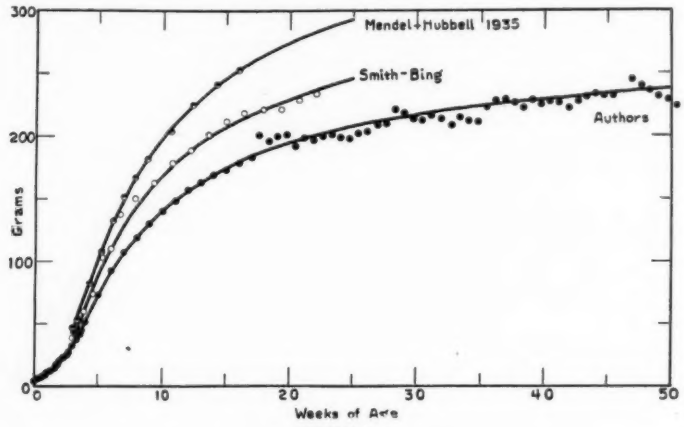

FIGURE 2

THE GROWTH OF WELL NOURISHED FEMALE RATS

(The authors' data are those of Tables 1 and 2, and Table 1 in Zucker, *et al.*, 1941.)

markedly cyclic growth having the same period as the oestrous cycles which was found in mice (Kopec, 1938; Adamska, 1938). Such growth cycles were also *not* reported by Evans and Bishop (1922) in their careful study of daily weights, food intakes and vaginal smears in the Long-Evans pied rat strain. Richter (1933) found such cyclic behavior in female rats in which the pituitary stalk had been injured, but not in normals.

Pre-Weaning Data

Such data are not included in our ordinary colony growth records, so we have no information on the pre-weaning growth of the particular rats of Table 1. To complete the curve we have supplied data on the growth of an entirely different set of animals (see Table 2). The two groups of rats should be indistinguishable; they were raised under identical conditions of colony diet and management, and were chosen from a large number which had been weighed up to 28 days of age using the same criteria as were used in selecting the rats of Table 1. The mean 28-day weights of the two sets of rats are the same, the standard deviations (σ) are close, as are the mean litter sizes (6.2 at birth and 5.5 at weaning for the rats of Table 1, 6.2 at birth, and 5.1 at weaning for the rats of Table 2) and the subsequent course of growth of the rats of Table 2, as far as it has been

TABLE 2
PRE-WEANING GROWTH OF FEMALES

Age days	N	Av. wt. grams	σ
0	27	4.84	0.36
2	13	5.94	0.64
4	13	7.1	0.58
5	14	8.3	1.49
6	10	9.5	0.50
7	30	11.0	3.24
9	19	12.8	2.14
11	18	15.0	1.90
12	14	17.9	2.81
13	12	17.8	1.01
14	35	20.6	3.51
16	12	23.6	3.23
18	14	24.9	2.49
19	26	27.4	3.69
21	27	32.3	3.47
23	19	37.7	4.32
25	18	41.4	3.67
26	16	44.9	5.51
28	36	51.4	4.93

carried, is entirely similar to that of Table 1. We therefore feel justified in combining the two sets of data. Thirty-six females are represented in Table 2, weighed at intervals of one to four days.

Variability

σ in both tables is the standard deviation $\sqrt{\frac{\Sigma d^2}{N-1}}$. The variability is partly due to individual fluctuations in weight, partly to inherent size difference among individuals which is maintained throughout life (see Figure 1). The fluctuations may in part be explained by the widespread incidence of respiratory infections in the colony at all ages. By this we do not mean the occasional severe illness often called rat pneumonia, for which animals are immediately discarded, but an ailment usually of brief duration something like the common cold, characterized by inflammation of or discharge from the eyes or nose, or just audible wheeziness. There may be a temporary marked growth inhibition, followed by rapid recovery. Variation in intestinal contents is not a large source of error in this species. The intestinal fill amounts to only a small percentage of the body weight (see data in Conner and Sherman, 1936; Sherman and MacLeod, 1925); and tends to "grow" as the body grows, probably as a reflection of the growth of the intestinal tract. Hourly fluctuations

are not large because rats eat more or less steadily. However, more eating is done at night than during the day, so all weighings were made in the morning.

Formulation

For simple equations which fit these data see the following paper.

Discussion

The Location of the Point of Inflection

Everyone is familiar with the data in the literature which place the point of inflection in the weight time curve at different positions in different species (see Pearl, 1924; Brody, 1925). This variably located point of inflection is the most important feature of growth curves for curve-fitting purposes; attempts to provide for it in rational growth equations inevitably influence the course of development of the theoretical side as well. The practical importance of nutrition in growth studies is brought out by the fact that the location of this most important feature is largely under nutritional control, at least in the two species chicken and rat. In 1928 Titus and Jull showed that improvement of a currently favored chicken ration by giving free access to sour skim milk caused the point of inflection to come at an earlier age without affecting the final size. Exactly the same shift is apparent in the growth curves of rats; as one improves the diet the point of inflection appears at a younger age and smaller weight. Figure 2 shows the characteristics of the growth of female albino rats on three different diets all probably approximately adequate for uninhibited growth. In our curve the point of inflection is located at three to four weeks (weaning). Pre-weaning data for the other two colonies are not available; we can only say that the point of inflection is not late. These three colonies incidentally show the range of inherent size in rats. Most other albino colonies are closer in size to ours, but the related Yale (Smith-Bing) and Storrs (Mendel-Hubbell) colonies have been systematically bred for size over a period of years (Mendel and Hubbell, 1935). Comparison of Figures 1 and 2 shows that the largest of our three females is about the same size as the mean for the Smith-Bing colony.

Figure 3 shows the delayed point of inflection found with suboptimal diets, comparing females of approximately the same inherent size. In Figure 3 Curve 1 is our growth curve. Curve 2 represents the growth of the Sherman colony females on the Sherman diet *B*

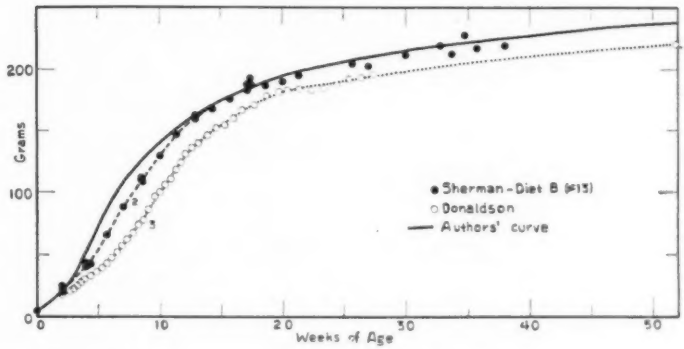

FIGURE 3

THE DELAYED POINT OF INFLECTION RESULTING FROM DIETS SUB-OPTIMAL FOR GROWTH

(Sherman, *et al.*, 1924, 1925, 1931). This diet, composed of two parts whole wheat, one part whole milk powder, and sodium chloride, is adequate for continued reproduction and colony maintenance but is not optimal, nor has it been adjusted for the most rapid growth (Sure, 1929-30; Russell, 1932; Campbell and Sherman, 1938; Van-Duyne, Lanford, Toepfer and Sherman, 1940; Kao, Conner and Sherman, 1941). The point of inflection is in the neighborhood of six weeks. The comparison of the Sherman data and our own is of direct interest because our colony derives from 93 rats (70 females, 23 males) obtained from Dr. Sherman during the years 1933 through 1936. The number is large enough, and was chosen from the Sherman colony sufficiently at random, so that these rats should have been a fair representation of the genetic characters of the Sherman colony. Placed upon our diet the rats of the new colony showed our characteristic growth curve from the beginning. Sherman's rats on diet *B* have not changed their growth curve between 1923 and 1940. The difference between the two curves therefore appears to be of nutritional origin. The two sets of rats are born with the same weight and the curves practically coincide from about 12 weeks on, but in the intermediate period the Sherman diet *B* will not support as rapid a growth as our diet does.

Curve 3 in Figure 3 represents the Donaldson data (Donaldson, *et al.*, 1906), with a point of inflection at 10 weeks. Donaldson's stock diet at that time has been described by Watson (1905):

> "Baker's bread, softened with skim milk, was the chief article of food. . . . Sunflower seed and cracked corn were always kept in the cages. Vegetables and meat were given once a week. Great care was taken to avoid surfeiting the rats. They were fed each morning between 9 and 10 o'clock."

An indication of the poor quality of this diet is found in the strain imposed on rats of this colony as a result of pregnancy and lactation, with physical exhaustion and temporary weight losses a usual occurrence.

During each lactation several females showed recurrent ulcers on the neck. When sufficiently well supplied with essential dietary factors rats can undergo up to 10 successive pregnancies and lactations with no rest between, and show no temporary weight losses or other adverse symptoms (Cole and Hart, 1938). The performance of the Donaldson rats could only have been called normal because the superior reproductive performance of which the rat is capable was not yet known.

The same situation is revealed by comparing the data published by Donaldson with those of King (1915*b*) on rats from the same colony and with the same final size. King used a better diet and the point of inflection is moved to a younger age and smaller weight. These data have been in the literature since 1915 without their import being noted.

The Relation between Diet and the Location of the Point of Inflection

To cause a point of inflection in the growth curve at any age experimentally it is only necessary to impose a restraint upon growth and later remove it; if the organism has not been significantly injured by the restriction it tends to recover and grow back towards the uninhibited growth curve, passing through a maximal growth rate in the process. One way to do this is to impose temporary dietary restrictions of various kinds. There is an extensive literature on the realimentation of rats after such temporary dietary restrictions (see Osborne and Mendel, 1916; Jackson, 1936, 1937, 1938; Clarke and Smith, 1938; McCay, Maynard and co-workers, 1935, 1939. See Stearns and Moore, 1931, for realimentation in children). McCay, Maynard, and co-workers produce a growth curve which is a series of steps, each with its point of inflection, by giving rats a basal diet which is adequate in all essential food factors but contains only enough calories to maintain weight, supplemented at intervals with a calory increment which causes the weight curve to climb the next

step. We have produced a slightly delayed point of inflection by separating the young from the mother at three weeks instead of four, without supplying any soft or liquid foods (in consideration of their limited dental equipment) to replace the mother's milk over this period of a week. To cause a deviation from the growth curve like that represented by Donaldson's data it would be necessary to impose a growth restraint beginning perhaps at birth and gradually decrease the severity of this restraint as the animals grow.

Now it is not at all unlikely that this is just the way certain inadequate diets fed *ad libitum* operate. The absolute daily intake of any factor fed at a constant level in a diet supplied *ad libitum* increases steadily with growth, and the absolute requirement may increase more slowly than the food intake, or actually decrease. This would be especially probable for factors largely built into the body, for the amounts so used per unit time decrease steadily just as the growth rate does. The evidence that this is so for protein seems quite clear from the reviews of Smith (1936) and Heuser (1941) for poultry, and from the detailed data on amino acid requirements of rats of Burroughs, Burroughs, and Mitchell (1940). The same is true of calcium and phosphorus, the realimentation also finding expression in bodily composition. Low levels in the diet of rats result in a low percentage of these elements in the body of the growing animal, but as the rats on the poor diets grow older they gradually approach the bodily composition of rats raised on higher levels (Sherman and Quinn, 1926, for phosphorus; Sherman and Booher, 1931, for calcium). The composition of the Donaldson diet suggests sub-optimal levels of protein and calcium.

Of course spontaneous realimentation will be impossible on serious deficiencies leading to secondary symptoms which constitute an added cause for retardation, nor is it possible for all food factors. The absence of spontaneous realimentation on diets mildly deficient in thiamin is evident from the work of Drummond (1938).

The Shape of the Growth Curve

In the rat, then, the inherent growth curve is characterized by great asymmetry with the point of inflection at 1/6 to 1/7 of the limit weight. Inferior nutrition can result in an artifically delayed point of inflection at as late as 1/3 to 1/2 of the final weight, and the recovery from the nutritional inhibition tends to produce a more

sharply defined knee or bend than is truly characteristic. It is extremely probable that other growth data which have been used for growth formulation have similarly delayed points of inflection, for spontaneous realimentation on certain kinds of mildly deficient diets should be a general phenomenon. It is unfortunate that detailed growth studies on diets embodying the extensive recent work on the nutritional requirements of farm animals have not been published. With chickens, for instance, the investigations carried out have centered on other nutritional criteria of greater practical interest—egg laying capacity, hatchability, shell quality, blood spots, etc.

In this connection the shape of the human growth curve is of interest. As usually presented there are two points of inflection: one in early infancy in the neighborhood of birth and the other during adolescence when about 2/3 of the final weight has been achieved. This second point of inflection is preceded by a long period of almost linear growth (Davenport, 1926; Brody, 1927*e*), which gives the curve such a unique appearance that Brody has suggested that human growth is different in its nature from other animal growth. Before such a conclusion is accepted it should be realized that these curves are usually made up by joining together several age categories of distinctly different nutritional background. Data for infancy and early childhood usually come from pediatricians who prescribe diets of relative uniformity and high quality for mother and child. They can be expected to be near optimal while those of older children and adults are very diverse and, in the aggregate, far from optimal (see, e.g., Stiebeling and Leverton, 1941). Davenport (1931) has pointed out that individual children have their adolescent point of inflection at very different ages and weights so that the average curve is not like that of any individual.

We are not suggesting that all differences in shape of growth curves, and specifically in the location of the point of inflection, are necessarily of nutritional origin, for they may to a certain extent arise out of variation in the inherent course of development (genetic, racial); however nutritional effects have in the past been disregarded far too much. It is difficult in view of the nutritional shift to say whether the location properly varies from species to species or not.

The Significance of the Point of Inflection

We can now compare the status of the point of inflection as based on the older data with its probable rôle in unhampered rat growth.

The most explicit statement with regard to its meaning derived from the Donaldson data and extended also to other fields is contained in several papers of Brody:

> "The point of inflection, then, seems to have significance in indicating: (1) the time of maximum velocity of growth, that is, the transition from increasing to decreasing growth velocity; (2) the age of puberty; (3) the lowest specific mortality; and (4) finally, it represents a point of reference for the determination of equivalence of age in different animals (and also equivalence of age in the growth of populations). The point of inflection should, therefore, be considered as a rather important growth constant." (1927*a*, p. 33.)
> ". . . animals differ enormously with respect to the position of the major inflection (puberty) in the growth curve. . . . The position of the inflection in the curve of a given species is probably related to the position of the species in the scale of evolution." (1927*d*, p. 19.)

In its main features this was reaffirmed (Brody, 1928) after Osborne and Mendel (1926) had pointed out that better growth data "may not be in harmony with some of the current interpretations of growth." Quite recently Backman (1938) has taken a stand similar to Brody's; he suggested that any 2 animals are of physiologically equivalent age when their respective growth rates and ages, expressed in units of the growth rate (maximal) and age characteristic of their points of inflection, are the same.

First of all, with regard to equivalence of age in different species and the position in the scale of evolution, it is obvious that judgment must be withheld until data for the several species or subspecies concerned are available which give at least relative assurance that the point of inflection as recorded represents a property of the animal and not a property of the diet which happened to be consumed. Similar considerations apply to the specific mortality rate. The weakness of the position regarding equivalence of physiological age at the point of inflection is probably best illustrated if we propose to apply it to a comparison of the three sets of data of Figure 3. In fact it is difficult to see any physiological similarity between our rats at three to four weeks just making themselves independent of the maternal food supply, with 1/6 to 1/7 of their final weight achieved, still having to double their weight before the vagina opens, and the Donaldson 10-week-old rats which have trebled their weight on an independent food supply, are sexually mature, and have attained 1/2 to 1/3 of their final weight.

The coincidence of the point of inflection with puberty is a fact for the Donaldson data, and may well hold for other rats reared on deficient diets. No one has however even suggested that normal sexual maturity sets in at four weeks, the region of maximum growth rate in the three colonies of Figure 2. A finding with regard to puberty which fits both the Donaldson data and the new data is that the vagina opens at 100 grams body weight (Anderson, 1932). On poor diets this weight is attained at a later age. The dissociation of time of puberty and maximum growth rate finds further support in at least some of the data assembled by Kaufman (1930). While both time of puberty and time of the maximal growth rate can be nutritionally affected their simultaneous occurrence is possible only on deficient diets. Normally they not only appear to have no such relation to each other but it is also clearly seen that the onset of sexual maturity, the region of 100 grams body weight, is not accompanied by any visible break or change in the consistent gradual decrease in growth rate.

Summary

New growth data for female albino rats from birth to 70 weeks of age on a diet near optimal for growth by current nutritional standards necessitate some revision of the older generalizations on the shape of the normal growth curve and the physiological significance of the point of inflection, and support with practical results the general expectations regarding the necessity of more attention to nutrition in growth studies.

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QUANTITATIVE FORMULATION OF RAT GROWTH

LOIS ZUCKER, LILIAN HALL, MARGARET YOUNG, AND THEODORE F. ZUCKER

Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City
(Received for publication, October 17, 1941)

Most students of growth postulate that growth is not a single unit process, but is the resultant of several cycles of growth; it is these several cycles for which relatively simple laws may be expected, while the growth as a whole will be governed by a more complicated function. The first step in any attempt to develop a rational formulation of our new data on the growth of rats on fully adequate diets (Zucker, *et al.*, 1941*b*) must therefore be concerned with the development of a point of view on this question of the unity of the growth process, for obviously the forms of the equations which can fit the data are entirely dependent upon how the data have been divided up into assumed cycles.

For discussions of cycles the reader is referred to Backman (1931); von Bertalanffy (1934, 1938); Brody (1927*a*, *b*, *c*); Courtis (1937); Davenport (1926); Needham (1931); Robertson (1923, 1926); Saller (1927); Schmalhausen (1926, 1929*a*, *b*); Robb (1929). A great many causes for cyclic growth have been suggested which would apply to the rat. There are the changes in environment and nutrition accompanying birth and weaning. There are the special changes and hormonal stimuli of puberty, and cycles have been referred to the temporary predominance of other glands of internal secretions as well. Considerable emphasis has been placed on hormonal activity as a source of cycles, because endocrine effects on growth are known, and the relative activity of the various glands is not constant throughout life. Distinctions are made among the processes of growth in size of cells, increase in number of cells, and increase in weight of non-cellular material. Some students of embryonic growth suppose a number of cycles connected with the successive development of various tissues—distinction being made both in the morphological sense and in the chemical sense.

Some of these have been applied in the various formulations worked out for the extensive data on rat growth assembled by Donaldson (1924); these are reviewed briefly in the following section in order to illustrate the ways in which cycles have been applied. Figure 1

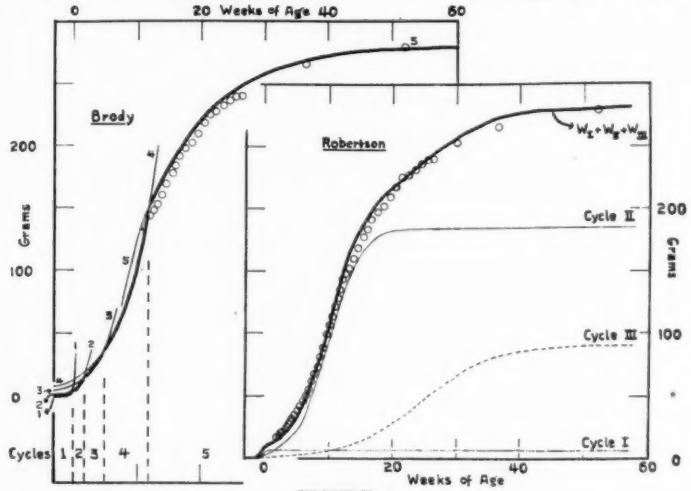

FIGURE 1

THE BRODY (CONSECUTIVE CYCLES) AND ROBERTSON (SIMULTANEOUS CYCLES) TREATMENTS OF THE GROWTH OF THE DONALDSON MALE RATS

The data points have been omitted in the graph of the Brody treatment up to 12 weeks of age so as not to obscure the cycle boundaries. The fit is very good in this region.

serves to illustrate the two kinds of cycle schemes which have been used—consecutive corresponding to a succession of growth processes separated in time, and simultaneous corresponding to a number of growth processes operating side by side, each contributing a portion of the total weight gains. It may be noted that while each simultaneous cycle extends throughout the life period, its major contribution to weight increase is localized in time, and it is in this period that biological associations for the cycle are expected.

FORMULATIONS OF RAT GROWTH

Robertson (1923). Chemical Theory. Donaldson Data. Age range 10-365 Days

Throughout this period $W = W_{\mathrm{I}} + W_{\mathrm{II}} + W_{\mathrm{III}}$, so that there are

three simultaneous cycles. W for each cycle is given by an equation of the form

$$ln \frac{W}{A - W} = Ak(T - T^*)$$

in which A is determined by the quality of the food supply, k is a specific reaction rate, and T^* a reference point for the time scale; W is weight and T is time counted from conception. There are thus nine adjustable constants which are assigned rational meanings. The scheme of cycles is worked out by applying the equation to the data, and no evidence for it independent of the requirements of a fit is offered.

Crozier (1926). Chemical Theory. Donaldson Data. Age Range 10-365 Days

From 10 to 95 days $W = W_I$; from 95 to 365 days $W = W_I - W_{II}$ (2 simultaneous cycles). W_I is given by the equation

$$ln \frac{A(K_2W + K_1)}{K_1(A - W)} = (K_1 + K_2A)T$$

$$W_{II} \text{ by } W = M_0 \frac{K_3}{K_4 - K_3}\left(\epsilon^{-K_3(T-95)} - \epsilon^{-K_4(T-95)}\right)$$

All K's are specific reaction rates. A and M_0 are the original supplies of two particular chemical substances involved in the postulated reactions as reagents. There are thus six adjustable constants given rational meanings, and one further adjustable feature in the location of the beginning of the second cycle. The second cycle and its approximate location are justified by the fact that W_{II} has a maximum at 150 days, where Donaldson noted a maximum in thyroid activity.

Brody (1927b). Chemical, Cellular-Population Theories. Donaldson and Stotsenberg Data

Age range conception (—22) to 365 days, divided into five consecutive cycles from conception to birth, birth to 13 days, 13 to 35 days, 35 to 80 days, and 80 to 365 days. In the first four cycles the weight is given by an equation of the form $W = A\epsilon^{-kT}$ in which k is a specific reaction rate and A is the initial weight. There are different values of k and A for each cycle. The weight in the last cycle is given by

$$ln(A - W) = -k(T - T^*) + lnA$$

in which k is a specific reaction rate, A, the final size, is associated with the initial quantity of chemical reagent and also with some sort of growth inhibiting agent produced by the growth itself (comparable to acid production in bacterial cultures), and T^* is a reference point for the time scale. The location of the boundary between the last two cycles, at 80 days, is justified by its association with puberty (see Zucker, *et al.*, 1941*b*). The other three cycle boundaries arise simply from curve fitting and are therefore additional adjustable features of the formulation.

Pearl (1924). Cellular-Population Theory. Donaldson Data. Age Range 10-365 Days

W throughout is given by

$$W = W_0 + \frac{A}{1 + \epsilon^{a_0 + a_1 t + a_2 t^2 + a_3 t^3}}$$

W_0 is the initial weight, A the final weight, t age from birth, and the other four constants are given no interpretation.

von Bertalanffy (1934, 1938). Surface-Volume, Chemical Theories. Donaldson Data

Age range 0-365 days, divided into two consecutive cycles, from birth to 70 days and from 70 to 365 days. W in both periods is given by an equation of the form

$$W = [A^{\frac{1}{3}} - (A^{\frac{1}{3}} - W_0^{\frac{1}{3}})\epsilon^{-kt}]^3$$

W_0 is the initial weight. $k = K/3$, where K is the rate of decrease in weight per unit caused by catabolic processes. A is the final weight, and is proportional to the cube of H/K, where K has the same meaning as before and H is the rate of increase in weight per unit surface, caused by synthesis. The same value of W_0 is used in both cycles, but there are two values of k and two values of A. The cycle boundary at 70 days is associated with puberty. This equation is to apply only to growth in which proportions and shape do not change. Prenatal growth is excluded, but post-natal growth is considered a sufficiently close approach to proportional growth.

Hatai (in Donaldson, 1924, and Hatai, 1911). Dynamical Theory. Donaldson Data

Age range 10-365 days, divided into two consecutive cycles with the boundary at 80 days. The weight in the first cycle is represented

by $W = a + bt + ct^2$ which has not been rationalized. Weight in the second cycle is given by $W = a \ln t + bt + c$ based upon the principle of least action.

Schmalhausen (1928, 1929a). Cellular Theory. Greenman and Duhring Data, and Stotsenburg Data (Donaldson, 1924)

Age range conception to 450 days divided into four consecutive cycles, conception to birth, birth to 101 days, 101 to 134 days, and 134 to 450 days. The period from 101 to 134 days is not fitted and is looked upon as a transition period associated with puberty. Weight in the other three cycles is given by equations of the form

$$\ln W = k \ln(T - 8) + C$$

or, as Schmalhausen calculates the specific growth rate from the original data, and fits this new variable as a function of time,

$$(T - 8)\frac{d \ln W}{dT} = k.$$

k is called the growth constant.

von Hoesslin (1930, 1932). Surface-Volume Theory. Data on the Growth of 1 Rat (King, 1916-17)

In the period 20 to 150 days weight is given by

$$\left(\frac{W}{A}\right)^{\frac{1}{3}} = \frac{t + t^*}{T'}\left[2 - \frac{t + t^*}{T'}\right].$$

T' is the length of the growth period. From birth to 20 days the equation does not fit the data, and the animal was killed at 150 days. The equation indicates that growth ceases at 150 days (when $t = T'$) and von Hoesslin considers that any further gains would represent fattening rather than true growth.

Growth Theories

Cellular

Attention is focused on the rate of increase in the number of cells rather than on the rate of increase in the weight of the entire organism comprising both cellular and non-cellular material. It is generally agreed that each type of cell has a basic reproduction rate and growth rate. Some emphasize the continued decrease in the number of cells which can reproduce as the organism grows (Schmalhausen, 1929*b*). Others emphasize the modifying effect of the con-

tinuously changing cellular environment and food supply on the basic rate (Brody, 1927*b*). By a natural extension of this latter viewpoint the growth of populations, whether of unicellular or of multicellular organisms, is considered part of the same problem of the reproduction of identical units in a changing environment. For discussion of the population aspect of growth see Brody, 1927*b;* Pearl, 1924; Courtis, 1937, and for criticism see Richards, 1934.

Chemical

The fundamental rate determining process is a chemical reaction. The growth equation is expected to take the form of the equation for a simple chemical reaction. For examples see the references cited above. For criticism see Murray, 1926*b;* Snell, 1929; Gray, 1928; Teissier, 1936.

Dynamical

This view proposes the treatment of growth by the laws regulating the exchange of energy in mechanical systems. For a most elaborate example which has not yet so far as we know been applied to rat data, see Wetzel, 1937.

Surface-Volume

Food is absorbed at surfaces. The larger the surface relative to the volume which must be supported by the food the more favorable are the conditions of food supply. The ratio of surface to volume is the fundamental determinant of growth. For examples see, in addition to the references cited above, Pütter, 1920; Fischer, 1928; for a pertinent objection see Gray, 1928, p. 269.

Application to Our Data: Adult Growth and Fat Deposition

The first question which must be answered in connection with any proposed cycle scheme is this: Does the biological event or process take place at or during the time, and in the manner, demanded by the proposed scheme? In most cases this question can be answered in reasonably satisfactory fashion, and one must then proceed to the more difficult question of evidence that the proposed event or process is the cause of a change in the fundamental manner of growth which demands a cyclic treatment. However there is one reason which has been given for dividing up rat growth data which can be definitely rejected for our data on the basis of the first question. Our data

show that weight continues to increase slowly as late as 70 weeks of age, and this same feature has been observed by other investigators. The weight increase from about 30 weeks on has not infrequently in the past been characterized as fat deposition, not true growth, and therefore as constituting a separate process not governed by the laws of true growth. (King, 1915; v. Bertalanffy, 1934; v. Hoesslin, 1932; Brody, 1927*d*, applies this idea to the growth of mice, and also appears to do so with some rat data, judging by a comparison of his tabulated fits with the original data.) However a considerable body of evidence goes to show that the growth of our rats even up to 100 weeks of age represents general body growth and not excessive fat deposition. This does not mean that the proportion of fat in the body of the rat may not increase normally with age. Fat is a normal functional component of the body. The proportion of fat in the body gradually increases as the animal grows, but this gradual increase accompanies early growth as well as later growth, being heterogonic to body weight (Needham, 1934). Such a slow increase in proportion is logically considered a normal accompaniment of true growth. This is in sharp contrast to the postulated abnormal obesity which sets in as the rats grow older and involves perhaps 10 to 20 per cent of the total body weight. The evidence may be summarized as follows. (*a*) The gross appearance of the rats does not suggest obesity such as one sees in an obese human being or dog, nor is there any appearance of obesity at autopsy. (*b*) The mean weight time curve shows an orderly gain in weight at a continuously decreasing rate (see Figure 2). This feature is also observed in the individual curves; there is no break at any point, and it would be difficult to say where one might suppose true growth to stop and obesity to set in. (*c*) Dawson (1925) found that the last closure of epiphyses of the long bones occurs as late as 1,400 days. This means that increase in weight by cell division can still occur at this age. Increase in size of skeletal frame is probably the safest indication that true growth is still going on. Substantiating Dawson, we find skeletal growth in our rats up to 100 weeks of age; this is shown in Figure 3, in which the log of the weight of the dry fat free femur is plotted against the log of the total body weight for individual females ranging in age from 24 to 750 days. (These data are part of a detailed unpublished study of the relative growth of the bone). The log log relation is the well known law of relative growth, or heterogony (Huxley, 1932; Needham, 1934), very

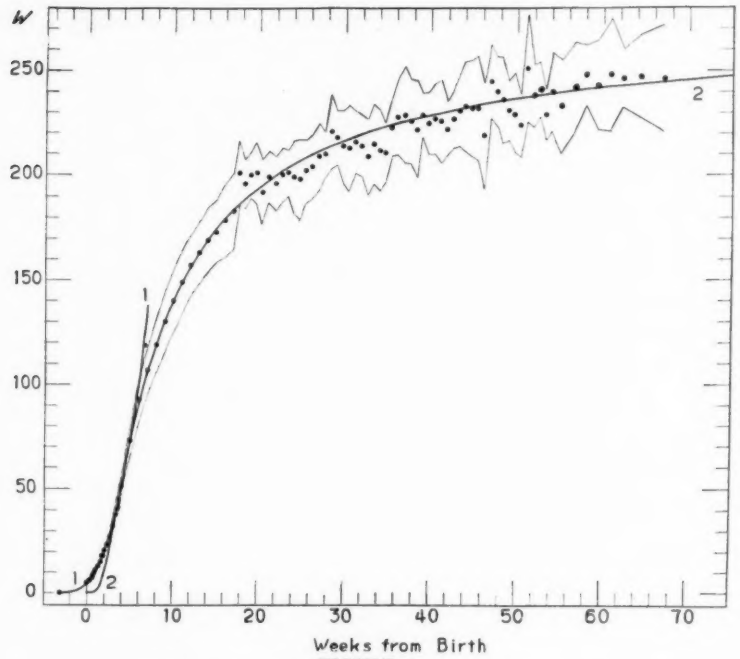

FIGURE 2

MEAN WEIGHT—TIME PLOT OF OUR FEMALE GROWTH DATA

The two curves are the equations $\log W = 2.88 \log T - 3.182$, T being days from conception, and $\log W_t = -\frac{2.84}{t} + \log 270$, t being weeks from birth. The fine broken lines on either side of the data points represent plots of $W + \sigma$ and $W - \sigma$; with a normal distribution, 2/3 of the individual weights should lie inside the band so defined.

widely applicable to the relative growth of parts of the body. The mean body growth in the three oldest age categories represented by weights of 237, 242, and 254 grams, is accompanied by femur weights of 423, 425, and 472 milligrams. Entirely comparable data show that the bone ash and the organic matrix of the bone also grow heterogonically to body weight from 24 to 750 days. Therefore true growth is taking place in this period. Furthermore the relation governing the growth of bone components relative to body growth characteristic of the young rapidly growing rat is maintained without a break in older rats; excessive fat deposition, which would affect the body weight

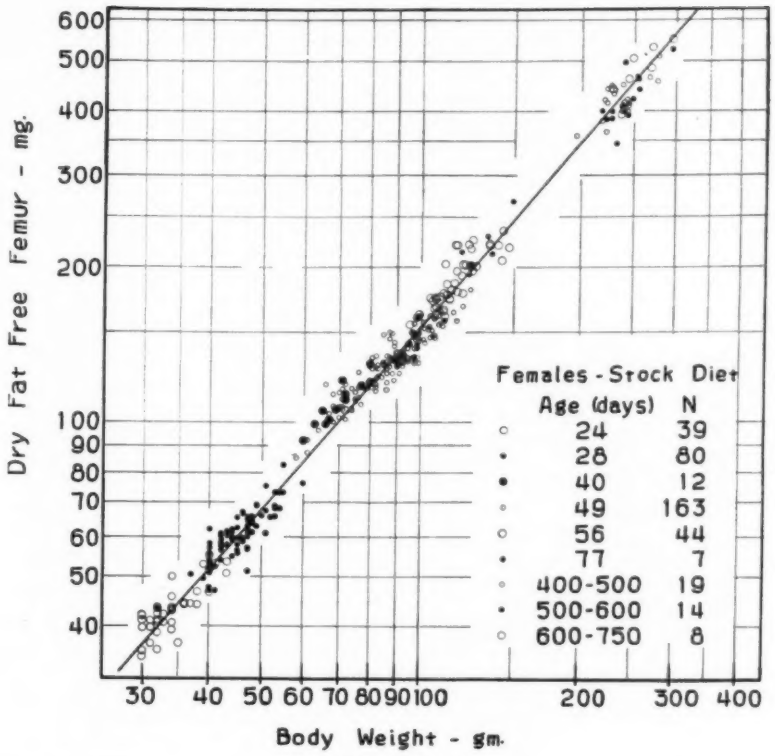

FIGURE 3

HETEROGONIC GROWTH OF THE FEMUR—PLOT OF LOG WEIGHT OF DRY FAT FREE FEMUR AGAINST LOG BODY WEIGHT FOR INDIVIDUAL FEMALES OF OUR COLONY

These are not the same animals as those of Figure 2 but they are in every way comparable. The line is the equation *log* (femur weight) = 1.188 *log* W — 0.189.

but not the fat free femur weight (or ash, or matrix) should cause the later points on the graph to be too low. It appears therefore that fat deposition, beyond that which is part of the growth plan extending over most of the life span, can be excluded in the data of Figure 2.

It is not impossible that obesity may set in later in life in rats when sexual activity has ceased, as Asdell and Crowell (1935) have suggested. Abnormal fattening cannot be categorically excluded at any age; it may set in as a result of definite pathological conditions, or,

as Johnson, Hogan, and Ashworth (1936) have shown, in young rats on inadequate protein intakes.

Evidence for Cycles

While in most other cases for which cyclic treatment has been proposed the existence of the process or event responsible for the cyclic treatment is conceded, this does not mean that the data must necessarily be divided up in the manner proposed. The general conceptual basis for any of the postulated growth cycles must be considered reasonable but not established. The fact that we can observe various events (e.g., birth, sexual maturity, involution of the thymus, etc.) during the life of the organism does not necessarily mean that these represent breaks or points of discontinuity in growth; the fact that we can observe various processes to which different names can be put (e.g., increase in number of cells, increase of size of cells, aging, etc.) does not necessarily mean that these are independent processes. The phenomena to which cycles are referred are usually necessary events or processes in normal growth which could be subsidiary to, and governed by, a unified process following a simple law. The situation is clearly different with regard to complications from nutritional deficiencies; here complexity necessarily results because the extent and number of the complicating deficiencies, arbitrarily imposed from outside, make a unified reaction impossible. As for the various *inherent* aspects of growth to which cycles have been referred, it is not obvious whether in principle it is better to treat some as independent to a first approximation, or whether the various processes are so closely linked that no analysis is possible.

If one accepted all the different possible causes of cycles which have been suggested as definite evidence that all these cycles exist and must be considered, it would have to be concluded that growth is so complex that even an approximately valid quantitative formulation is out of the question. Many suggested causes for growth cycles are difficult to locate in time, and there are so many possible causes that there is scarcely a period in the life of the organism for which one cannot, by making reasonable assumptions, postulate a separate growth cycle. The cycle theory applied to the fullest extent without rational substantiation should be able to make any equation, of whatever quality, fit almost any set of data, whether suitable or not, for it offers an almost limitless reservoir of adjustable features. Some

other universal curve fitting schemes such as the power series are quite properly viewed with suspicion because the way in which the adjustable features are introduced is difficult to reconcile with biological mechanisms (Brody, 1927*b*). There is unfortunately no such natural check on the free use of growth cycles.

The greatest difficulty in attempts to formulate growth is to avoid letting the general concept of cycles degenerate into a mere adjunct to empirical curve fitting, and if one is inclined to accept many of the proposed cycle causes as probable one might as well give up the idea of ever working out general laws of growth. This is one possible logical position. At the opposite extreme, just as logical, is the position that no cyclic analysis is fully justified, and that therefore none must be made; the data must be fitted whole, or not at all. It must be granted that no one who now supports this or that scheme of cycles would quarrel with one *simple* law successful in fitting the entire growth period, if such could be found. Much ingenuity has so far failed to develop such a law (and the cycle theory was introduced to explain this failure), but the whole question must now be reopened in consideration of the unsuitable character, and distorted form, of the older growth data (Zucker, *et al.*, 1941*b*). We feel that an intermediate position, just as logical but more realistic, would recognize the possibility of cyclic growth but would demand more detailed and objective evidence in support of any *particular* cyclic scheme offered.

Acceptable evidence cannot in any way be based upon the exigencies of curve fitting with a favored growth equation. No growth equation so far suggested is sufficiently well founded in theory to stand on its own feet—all must be judged by their relative success in fitting data which can be considered suitable. Such a growth equation is therefore not a suitable tool for working out the correct analysis of the life period into growth cycles, for it is a foregone conclusion that a fit will result whether the formulation has any significance or not (see Brody, 1927*b*, Courtis, 1937, and Robertson, 1923, for examples of this procedure using three different equations.) It has been suggested that growth cycles will make themselves known by bumps, breaks, or points of inflection in graphs of growth data—in the plot of specific growth rate, or growth rate, against time (Saller, 1927; Backman, 1931; Brody, 1927*b*), or in the plot of log weight against time (Brody, 1927*b*), or of $T \frac{dlnW}{dT}$ against time (Schmalhausen,

1929*a*). Here there is the difficulty that such features may be expected to appear as a result of experimental error quite independent of the existence of cycles—illness, nutritional deficiencies, differences in the size distribution or other growth characteristics of the individuals making up the averages at different points in the graph, etc. And if one establishes the validity of such a feature in the data, the interpretation of this feature as the boundary or center of a growth cycle involves certain assumptions about the functional form of the law. What will decide whether the true growth law is or is not characterized by the observed bump or break? These assumptions are similar in kind if not in degree to the serious one to which we have already referred of using a particular assumed equation to work out the cycle scheme.

Acceptable evidence in support of a cycle scheme must be based upon independent biological evidence, going far beyond the mere suggestions described above in the introductory remarks. It should at least be sufficiently quantitative to indicate the form of the cycles concerned (e.g., consecutive or simultaneous cycle type) and their location in time, and to argue a certain degree of independence in operation of the processes which are to be independently formulated. Having explored all available evidence one can only weigh relative probabilities and the result, whether it is a one-cycle scheme or a multicycle scheme, will be a hypothesis which must like any other hypothesis be judged by its success both in fitting representative data and in the kind of general clarification and productivity of ideas which are to be expected from a valid approach to any scientific problem.

A Proposed Analysis of Rat Growth Into Phases

We have already indicated reasons for separate treatment of pre- and post-weaning growth connected with the status of the food supply (Zucker, *et al.*, 1941*b*); the food intake of the nursling is partly determined by the number of litter mates and the lactating capacity of the mother, while that of the rat after weaning is entirely under its own control. There is another set of reasons for the same procedure which seem to us to have considerable force. Growth can be analyzed into two processes variously designated as development and growth, or histo- and auxano-differentiation; we postulate that the effects of these two processes upon weight increase are sufficiently independent as to suggest separate treatment. The terms histo-

and auxano-differentiation are due to Huxley (1932), and the following discussion is partly based upon his presentation. Huxley was principally concerned with the question of independent phases of the growth of organs relative to the growth of the body as a whole. The general idea that a change in status of the food supply at weaning may be coupled with some sort of change in the fundamental manner of growth for developmental reasons has been suggested by MacDowell, *et al.* (1930).

The two processes appear to be decidedly different in their manifestations, relatively independent in causation, and normally partly separated in time in many species. The organism begins as an ovum, structurally remarkably similar in different species, and proceeds by differentiation of new and different types of tissue with remarkable changes in form and proportions towards the recognizable pattern of the species—this is histo-differentiation. During this time, and later, the various parts of the organism increase in size—this is auxano-differentiation. The rather complete independence of the two processes is shown by the fact that rats placed at birth on such a limited intake of milk that they just maintain their weight go through, with but slightly delayed rates, the regular sequence of normal bodily development or histo-differentiation (Jackson, 1932; see also Gudernatsch, 1934 and 1936-37, for work on frogs); thus histo-differentiation continues when auxano-differentiation is entirely inhibited. In some species undergoing metamorphoses the extensive histo-differentiation leading to a marked change in form is normally accompanied by no increase in weight, or auxano-differentiation. With higher animals histo-differentiation is mainly compressed into embryonic and early post-natal life, and there is a long later period of relatively pure auxano-differentiation, with the animal increasing in weight but adhering closely to its already established pattern, with change in proportions small and governed by a simple law (heterogony). Other species may have several periods of auxano-differentiation separated by intervals of intensive histo-differentiation.

A number of considerations would lead to the selection of 3-4 weeks as an age after which no very significant amount of histo-differentiation takes place, and before which histo-differentiation dominates the picture. Histo-differentiation is by no means complete at birth, the rat being born in a very undeveloped state. The following details of the developmental history of the rat are of interest:

> Eyes and hearing start to function at 2 to 2.5 weeks of age; up until then the differentiation of these organs is incomplete. The rat is born completely hairless; hair coverage first appears at about two days of age in certain restricted areas, and continues to develop for some days thereafter. Many centers of ossification not yet established at birth appear during the first three weeks; these include the caudal vertebrae, some of the phalanges and small bones in the skull. Enamel and dentine formation of the incisors begins at about birth; the incisors erupt at 8-10 days, and the first and second molars at 19 and 21 days. The third molars erupt at 35 days, completing the dental equipment. The period from one to four weeks is one of extensive myelinization of nerve sheathes of the central nervous system. (Jackson, 1932, and Donaldson, 1924, pp. ix, 14-20, 37, 52, 117-120, 141, 151).
>
> " . . . in the rat nearly all growth after weaning (except bones, skin and hair) is by the enlargement of elements already present. . . . MacCallum (1898) and Schiefferdecker (1909, 1919) have disclosed the fact that during fetal life the number of muscle bundles becomes definitive. Moore's work (1930) on the kidney indicates that the same situation obtains here. Allen (1912) has studied the cessation of mitosis in the central nervous system of the white rat and reports that it is over by 25 days and that most of it is over long before that." (Moment, 1933).

Bodily proportions are very different at birth from those of maturity. By three weeks of age however the bodily proportions including the length of the tail are not remarkably different from those of the adult (Freudenberger, 1932). We do not mean to say that the rat's proportions are fixed at three weeks, but subsequent change is very gradual, whereas between birth and three weeks changes are rapid and very noticeable, so that the change concerned is of a very different order of magnitude.

> "The critical period in the chemical growth of the rat occurs at about the time of weaning (20-30 days). At this point the total solid, fat, ash and creatine have either reached or closely approximated 'chemical maturity.' . . . It is . . . possible that rats at this age are undergoing a metabolic as well as a chemical 'crisis.'" (Chanutin, 1931).

There remains the matter of the development of the reproductive system which does not reach a functional state in either sex until 6-7 weeks of age (Anderson, 1932). This final maturing process differs somewhat in nature from the previous developmental changes. Histo-differentiation is essentially age determined, and fairly independent of weight as determined by auxano-differentiation; this fact

is a principal reason for the separate formulation of growth characterized by extensive histo-differentiation and growth characterized by pure auxano-differentiation. But as we have already noted (Zucker, *et al.,* 1941*b*), puberty in the normal female does not display this independence of the state of auxano-differentiation, for it is associated with weight rather than age. Any procedure which slows up growth delays puberty. Evans (Evans and Bishop, 1922, 1923) has shown this for deficiencies of various essential food factors. Asdell and Crowell (1935) showed it for the limitation of weight gain by caloric restrictions; rats kept at 40 grams for some time and then allowed to gain very slowly did not become sexually mature until they were one year old, when they had reached an average weight of 84 grams. All of this suggests that the final attainment of sexual maturity is not determined by the relatively independently operating process of histo-differentiation, but is controlled by the rate of auxano-differentiation of the animal. This idea is very much supported by the finding (Cole, 1937) that a single injection of gonadotropic hormone at or after 21 days of age, *and not before,* brings about ovulation in the female within 72 hours; these rats in spite of their small size are able to undergo normal mating and pregnancy with litters of normal young born at term. The analysis of the period in the normal sexual cycle at which this hormone is liberated ordinarily shows that the normal cyclic process culminating in ovulation begins immediately after the hormone injection in the young rats (Cole, 1936). These results indicate that the essential histo-differentiation of the female reproductive system has reached a remarkably complete state at three weeks of age and the functional state, which can be induced at any age thereafter by experimental procedure, normally awaits the attainment of suitable size. We have no evidence on the comparable state of affairs in the male. The period from roughly three weeks to 100 grams weight in the female is therefore of the nature of a final transition from growth dominated by histo-differentiation to pure auxano-differentiation. During this period there is the final maturing of the reproductive system, and it is indicated that the thymus reaches its maximum size and begins to involute (Donaldson, 1924).

Equations Fitted to the Two Phases

Each phase is fitted very closely by a simple equation:

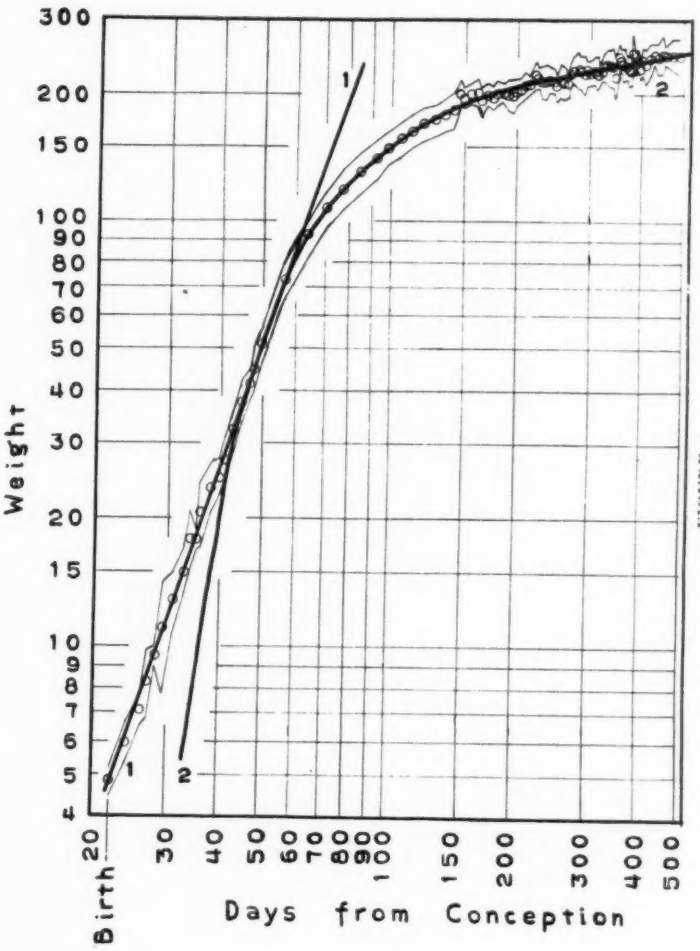

FIGURE 4

Log Weight—Log Time Plot of Our Female Growth Data

Straight line 1 and Curve 2 are the equations given in the legend to Figure 2. The fine broken lines represent plots of $log\ (W + \sigma)$ and $log\ (W - \sigma)$.

$$\log W = K \log T + \log C, \text{ or } W = CT^K \text{ pre-weaning}$$

$$\log W = -\frac{k}{t} + \log A, \text{ or } W = A\,(10^{-k/t}) \text{ post-weaning}$$

K, C, k and A are positive constants, T is age from conception in

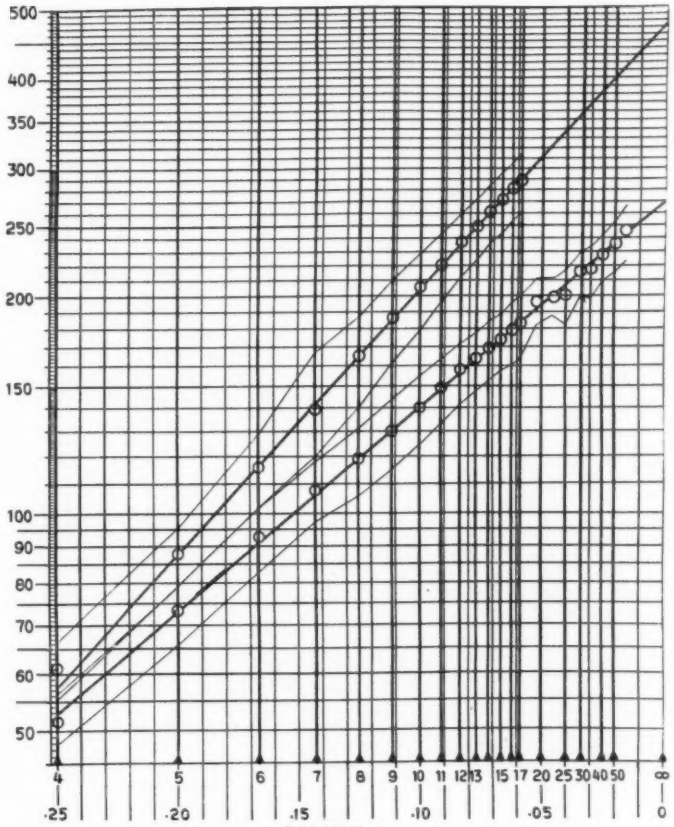

FIGURE 5

POST-WEANING GROWTH OF MALES AND FEMALES ON A LOG WEIGHT—RECIPROCAL TIME PLOT. THE 2 SCALES FOR THE ABSCISSAS ARE WEEKS AND 1/WEEKS

The straight line for the females represent Equation 2 of Figures 2 and 4. The period from 17 to 70 weeks occupies only 0.2 of the total space available in this graph, as against 0.5 on the log scale of Figure 4 and 0.8 on the linear scale of Figure 2. Therefore in order to avoid congestion in this graph the many data points for females in this period have been combined into a smaller number of points, each representing about 100 animals. These means on larger numbers follow a smoother course.

days, and t is age from birth in weeks. Figures 2, 4, and 5 exhibit the fit of the equations to the data. Figure 4 is a log log plot on which the first equation plots a straight line, and Figure 5 is a log reciprocal plot on which the second equation plots a straight line. The logarithmic weight axis common to Figures 4 and 5 is preferable to the linear axis of Figure 2 since it provides a more favorable distribution of the range of variation of the individual weights around their means in a band of approximately constant width. The first equation is well known, having been used for the embryonic growth of chickens (Murray, 1926*a;* Schmalhausen, 1927), guinea pigs and mice (MacDowell, *et al.,* 1927), and for the post-natal pre-weaning growth of mice MacDowell, *et.al.,* 1930). The particular form of the second equation has not to our knowledge been used before for growth. It and the log log equation are however both special cases of the general equation

$$log \frac{dlogW}{dT} = k_0 + k_1 log\ T + k_2 log^2\ T$$

applied by Backman (1931) to growth data, with his k_0 equal to log (.4343 K) and log k respectively, with k_2 equal to zero, and with $k_1 = -1$ in the log log equation and -2 in the log reciprocal equation.[1]

The Transition Period

Figure 4 (and less plainly Figure 2) shows the considerable overlapping of the two equations. The data from three weeks to about 100 grams are fitted equally well by the overlapping segments of both equations and thus display the characteristics of both phases of growth. There is a continuous gradation from one phase to the other, with no fixed point which marks a boundary between phases. The point of inflection is located within the transition period for both sexes. The data are in good agreement with the location of the point of inflection calculated from the sigmoid log reciprocal equation fitted to them—that is at 0.135 A for both sexes, or at 3.3 weeks for the females and 4.2 weeks for the males.

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[1]It should be noted that another equation $log \frac{dW}{dT} = k_0 + k_1 log\ T + k_2 log^2\ T$ which differs from the above equation by $log\ W = 0$ is also used by Backman in this paper, more or less interchangeably, and this second equation is called *the* Backman equation in two later papers (Backman, 1938) on the mathematical properties of his equation. The log reciprocal equation has no connection with this second equation, and the mathematical characteristics of this second equation developed by Backman in the 1938 papers in no way apply to the log reciprocal equation.

The gradual transition from one equation to the other covers the same period as the final transition from histo-differentiation to auxano-differentiation, the final maturing of the reproductive system, and the time necessary for the adjustment from the maternally determined food intake to a food intake fixed by the requirements of the organism. This analysis of rat growth into two phases with a relatively long transition period partaking of the characteristics of both phases is suggested not in the sense that it follows necessarily from the available evidence, but as a reasonable possibility. We do feel that the evidence assembled in its favor is both more extensive and more compelling than that put forward in support of any other such analysis, and the formulation as a whole is low in the number of adjustable constants. The practical advantages of being able to record the data directly in straight line form are striking.

Effect of Litter size Upon the Fit

We find an inhibiting effect upon growth produced by raising our rats in large litters which indicates that the food supply of the nurslings is restricted. MacDowell, *et al.* (1930) pointed this out for mice; they also found that the growth of the nurslings approaches the log log equation only as the milk supply is greatly increased, either by supplying additional foster mothers or by systematically reducing the number in the litter as the lactation period progresses. According to Smith and MacDowell (1930) the difference in weight established at weaning time between mice raised in small litters and in large litters persists to a considerable age. We find on the contrary that our rats raised in large litters show no greater deviations from a log log equation than do those raised in small litters. We customarily limit litter size at birth, but have raised some large litters specifically to elucidate this point. Figure 6 shows one example—the growth of the six females of a litter of 11 (11 born, 11 weaned). It will be observed that the log log line for these data is of lower slope than the line for the data of Figure 4 on animals raised in smaller litters; the rats in the litter of 11 were at first larger, probably because of larger inherent size, but gradually fell behind and were considerably lighter at weaning. As far as post-weaning growth is concerned, animals raised in large litters are too small at weaning for the log reciprocal equation fitted to the subsequent course of their growth, and, strangely enough, those raised in small litters are too heavy, and

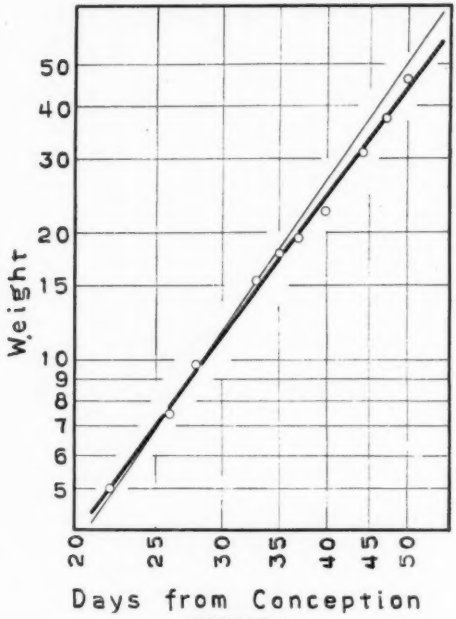

FIGURE 6

LOG WEIGHT, LOG TIME PLOT OF THE MEAN GROWTH OF 6 FEMALES RAISED IN A LITTER OF 11

The heavy line is fitted to these data. The light line is the line (1) of Figure 4.

come down onto the log reciprocal equation from above. Contrary to MacDowell's findings, the largest differences are fully made up by about seven weeks of age (after four weeks of eating stock diet). In line with the discussion in the preceding paper, we suggest that the differences between MacDowell's results and ours are not primarily species differences, but are quite reasonably explained on the basis of differences in the nutritional quality of the stock diets used.

Comparison of Figure 6 with the findings of MacDowell, *et al.*, and those of Sure (1940) provides an illustration of the relative simplicity of the intrinsic growth control mechanisms as against the complexity introduced by arbitrarily imposed extrinsic nutritional disturbances.

SUMMARY

The log of the weight of well-nourished rats plots a straight line

against the log of conception age, from birth to six weeks, and against the reciprocal of birth age, from three weeks on (data to 70 weeks). The period of transition (three weeks to six weeks, or 100 grams weight in the females) where both relations hold equally well, corresponds to: (*a*) the transition from intensive histo-differentiation to auxano-differentiation (terminology of Huxley) and the final attainment of sexual maturity, and (*b*) the transition from a maternally restricted food intake to a food intake determined by the growth requirements of the organism itself. It is quite probable that the end of this transition period is determined for the individual by weight rather than age.

Data are presented on the growth of the rat femur relative to body growth from 24 to 750 days of age. The excellent straight line heterogonic relation without a break over this entire period shows that the continued body growth of these rats is true growth and is not due to excessive fat deposition.

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THE EFFECT OF DIFFERENT DOSE LEVELS OF GROWTH HORMONE ON THE TIBIA OF YOUNG HYPOPHYSECTOMIZED FEMALE RATS

EDWIN A. KIBRICK, HERMANN BECKS, WALTER MARX, AND HERBERT M. EVANS

Divisions of Dental Medicine and Anatomy, the Institute of Experimental Biology, and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco and Berkeley, California
(Received for publication, October 28, 1941)

A. INTRODUCTION

Stimulation of skeletal growth after administration of growth hormone has been adequately demonstrated by a variety of methods, i.e., histologically (Handelsman and Gordon, 1930; Silberberg and Silberberg, 1939; Freud, Levie, and Kroon, 1939; Ross and McLean, 1940; Ray, Evans, and Becks, 1941*a*) and roentgenographically (Freud, Levie, and Kroon, 1939; Mortimer, 1937). However, all of the factors involved in this reaction have not been studied. The influence of age on the response of bone growth to growth hormone has been investigated by a number of workers (Handelsman and Gordon, 1930; Silberberg and Silberberg, 1939; Freud and Dingemanse, 1940; Ray, Evans, and Becks, 1941). The epiphyseal cartilage has been described as the localized point of attack by the Dutch group of investigators (Freud, Levie, and Kroon, 1939). Others (Handelsman and Gordon, 1930; Silberberg and Silberberg, 1939; Ray, Evans, and Becks, 1941*a;* Ray, Evans, and Becks, 1941*b*) state that all the histological elements of the skeletal system respond to growth hormone. Stimulation of skeletal growth has been described as a more consistent response to growth hormone than body weight gain (Freud, Levie, and Kroon, 1939; Ross and McLean, 1940; Ray, Evans, and Becks, 1941*a*). Yet no precise study of the relationship between progressive doses of growth hormone and the stimulation of bone growth has been made.

The work of Freud, Levie, and Kroon (1939) and Ray, Evans, and Becks (1941) clearly point to the young hypophysectomized rat as the animal of choice, since the first essential in such a study is

great sensitivity and wide latitude of response. As a further refinement to this study, a highly purified (cysteine-treated) growth hormone fraction was employed (Fraenkel-Conrat, Meamber, Simpson, and Evans, 1940).

The index of growth hormone activity used in this experiment was the thickness of the proximal epiphyseal cartilage of the tibia as measured by an eyepiece micrometer. On the basis of the work of Ray, Evans, and Becks (1941*a*) and subsequent work in this laboratory (Kibrick, 1940), the following premises were accepted as reason for the choice of this quantitative index.

Hypophysectomy rapidly initiates a loss in the dimensions of the epiphyseal plate, despite the fact that growth of cartilage and bone may continue for a short time in the young animal after the removal of the pituitary. This loss in thickness reflects the initial disturbance of the equilibrium that normally exists between chondrogenesis and osteogenesis. Administration of growth hormone rapidly restores the dimensions of the cartilage plate by stimulating first, chondrogenesis and then osteogenesis until an equilibrium is re-established.

B. Materials and Methods

In an effort to control the influence of age, young female rats of identical age were used throughout this experiment. Littermates were used wherever possible. Following weaning, the animals were maintained on our stock diet (Evans, No. XIV) until the 26th day. At this time, 30 of the 33 animals were hypophysectomized and all animals had wet Diet I (McCollum) added to their food.

After a postoperative interval of 12 days, the 30 hypophysectomized animals were divided into five equal groups, one control and four experimental. The experimental groups were given daily intraperitoneal injections for four days of a cysteine-treated globulin growth hormone fraction (Fraenkel-Conrat, Meamber, Simpson, and Evans, 1940). This preparation which had been previously standardized on a body weight gain response of hypophysectomized rats contained approximately 23 growth units per mg. (Marx, Simpson, and Evans, 1941). The minimal effective dose as determined by the same method was 0.043 mg./daily/10 days.

The preparation was subjected to the following tests for contamination. Daily doses of one mg. were injected into young hypophysectomized rats for 10 days and histological examinations were made of

the adrenals and ovaries. Some evidence of adrenocorticotropic activity was found but no signs of interstitial cell stimulating hormone were present. Daily doses of five mg. over a 4-day period in squabs revealed changes in the thyroid gland that indicated a trace of thyrotropic hormone. The same dose caused no crop gland reaction in these squabs so that the preparation can be considered free of lactogenic hormone.

Approximately 40 micrograms of this growth hormone fraction were necessary to produce the minimal significant effect on body weight increase in the 10-day test described by Marx, Simpson, and Evans (1941). The lowest dose chosen for this experiment was half of this amount while the remainder were multiples. Preliminary experiments on young hypophysectomized rats had established the temporal sequence of the epiphyseal cartilage response to "acute" doses of a growth hormone (globulin) fraction (Kibrick, 1940). Observations made at the 2nd, 4th, 6th, and 8th day following the start of daily intra-peritoneal injections one week after hypophysectomy showed a rapid increase in cartilage width up to the 4th day with a leveling of the response beginning at the 6th day (Figure 1).

Since the purpose of our study was to observe differential responses

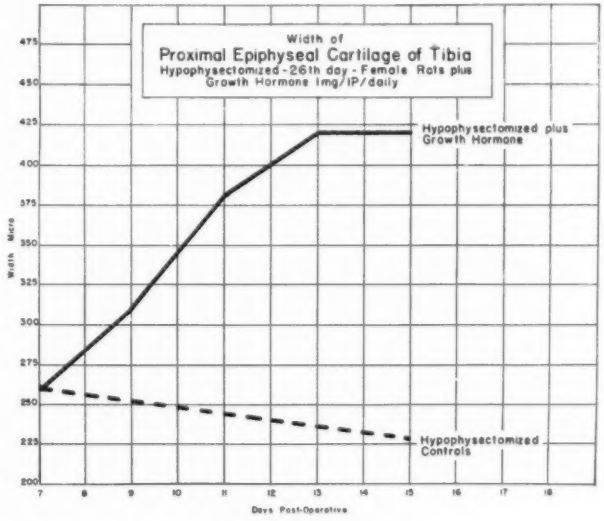

FIGURE 1

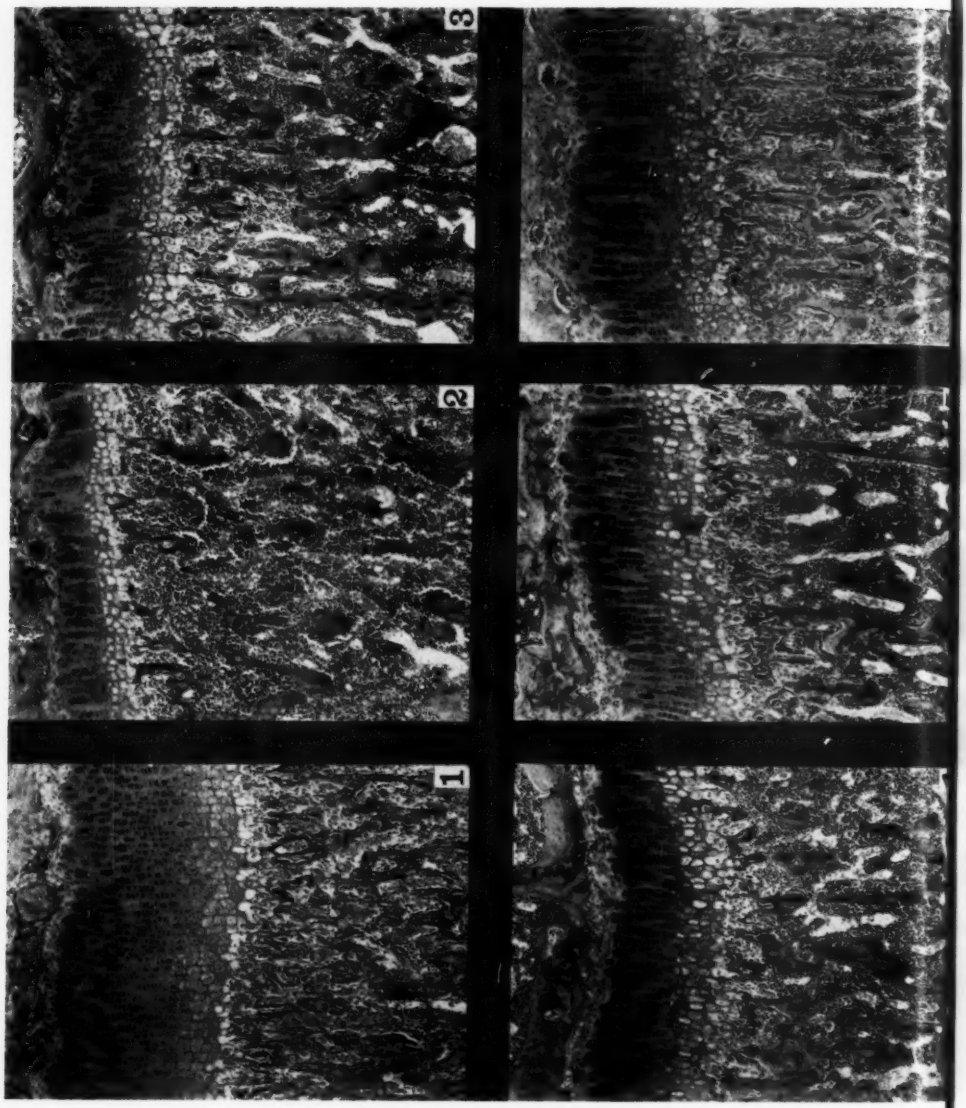
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FIGURE 2

CENTRAL SAGITTAL SECTIONS OF PROXIMAL EPIPHYSEAL CARTILAGE OF RIGHT TIBIA
(Hematoxylin and eosin, 10 μ x 100.)

1. Normal control, autopsied on 42nd day of age.
2. Hypophysectomized control, operated on 26th day, autopsied on 42nd day.
3. Hypophysectomized plus growth hormone, operated 26th day, injected from 38th day with 0.020 mg./*IP*/daily to autopsy on 42nd day.
4. Hypophysectomized plus growth hormone, operated 26th day, injected from 38th day with 0.040 mg./*IP*/daily to autopsy on 42nd day.
5. Hypophysectomized plus growth hormone, operated 26th day, injected from 38th day with 0.80 mg./*IP*/daily to autopsy on 42nd day.
6. Hypophysectomized plus growth hormone, operated 26th day, injected from 38th day with 0.160 mg./*IP*/daily to autopsy on 42nd day.

to graded dose levels, and the width of the epiphyseal cartilage was taken as our index, it seemed necessary to make our measurements at a point of maximum rate of response. Therefore, the effects of the different dose levels employed were studied after an injection period of four days.

The right tibia of each animal was decalcified in acid alcohol, imbedded in nitrocellulose and sectioned serially in a sagittal plane at 10 micra (Kibrick, 1941). Routine staining with Harris hematoxylin and eosin was used. Central sections were examined and measurements of the proximal epiphyseal cartilage were made with a calibrated eyepiece micrometer.

C. Results

The effects of hypophysectomy on the epiphyseal cartilage of a young rat are adequately described by Freud, Levie, and Kroon (1939) and Ray, Evans, and Becks (1941). In brief these changes are: atrophy of the cartilage plate; shrinkage plate; shrinkage of the cartilage cells; inactivity of the erosion zone; resorption of the diaphyseal trabeculae without increase in the number of osteoclasts; and increase of fat deposition at the expense of the myeloid elements of the marrow. There are certain minor differences in our illustrations that are due to differences of age and post-operative interval. The histological appearance of our hypophysectomized controls is well shown in Figure 2.

Examination of the tibias of animals treated with growth hormone revealed a progressive stimulation of epiphyseal cartilage proliferation correlated with the graded doses used, Plate I.

The measurements of the epiphyseal cartilage are listed in Table 1. For purpose of comparison the body weight gain during the 12-day post operative interval and the 4-day injection period are also presented. The cartilage width response follows the level of hormone administered more closely and with less variation than the gain in body weight. The difference between the means of Group II (hypophysectomized controls) and Group III (+0.020 mg. growth hormone) the lowest dose used, is markedly significant (4.98).[1]

Groups III to VI (20, 40, 80, and 160 micrograms, respectively) are not separated significantly but display a tendency toward an increased response in the same direction. It may be noted that if

[1]Ratio of significance.

TABLE 1

Group	Animal number	Initial weight gms.	Wt. gain (gm.) p.o. 12-day interval	Wt. gain (gm.) on injection 4-day period	Width of epiphyseal cartilage (μ) Prox. Tibia	Difference between Means
I Normal controls	GH4828	65	47	18	437	
	B4762	66	44	20	421	
	GH4779	63	47	12	421	
			$m = 46$	$m = 16.6$	$m = 426$	
II Hypophysectomized controls	GH4809	64	4	0	234	
	B4763	70	0	2	234	
	BH4867	65	2	—3	172	
	GH4769	48	12	1	250	
	W4770	57	5	3	234	
	BH4786	65	0	0	234	
			$m = 3.8$	$m = 0.5$	$m = 226 \pm 11.2$	
						67 ± 13.4 (4.98)*
III Hypophysectomized + *0.020 mg./1cc* growth *h./IP*/daily 4 days	BH4834	59	6	3	265	
	B4761	55	9	6	312	
	W4765	58	5	5	281	
	W4776	61	5	4	312	
	W4784	62	4	0	296	
	BH4794	61	4	4	296	
			$m = 5.5$	$m = 3.6$	$m = 293 \pm 7.4$	
						27 ± 14 (1.89)*

TABLE 1 (*continued*)

Group	Animal number	Initial weight gms.	Wt. gain (gm.) p.o. 12-day interval	Wt. gain (gm.) on injection 4-day period	Width of epiphyseal cartilage (μ) Prox. Tibia	Difference between Means
IV Same as above with *0.040 mg.* growth *h.*	B4823	54	5	1	359	
	G4827	64	8	8	328	
	G4843	59	6	1	281	
	B4760	67	3	6	343	
	W4783	57	0	3	296	
	BH4785	60	2	6	312	
			$m = 4$	$m = 4.1$	$m = 320 \pm 11.9$	
						17 ± 14.9 (1.15)*
V As above with *0.080 mg.* growth *h.*	G4824	62	4	8	374	
	G4825	66	2	4	328	
	G4842	58	3	4	328	
	B4764	63	2	4	312	
	W4781	65	3	2	328	
	W4782	62	2	4	359	
			$m = 2.6$	$m = 4.3$	$m = 337 \pm 9.2$	
						26 ± 20.2 (1.31)*
VI As above with *0.160 mg.* growth *h.*	G4813	59	2	5	374	
	G4817	62	3	3	296	
	G4844	58	2	9	406	
	GH4846	55	0	10	390	
	B4766	60	4	7	328	
	BH4796	58	7	4	390	
			$m = 3$	$m = 6.3$	$m = 363 \pm 17.3$	

*Ratio of significance.

the difference between the means of Group III and V is taken, the result, 44 ± 11.8, is highly significant (3.73).[1] The same procedure for Groups IV and VI results in a difference of 43 ± 21 which is relatively significant (2.05).[1]

Thus the statistical analysis of the cartilage width response reveals that either the doses are not sufficiently separated, so that an overlapping of groups results, or the number of animals was too small to establish the separation of each group.

A comparison of the cartilage response with body weight response under the conditions of this experiment is shown in Figure 3. Here

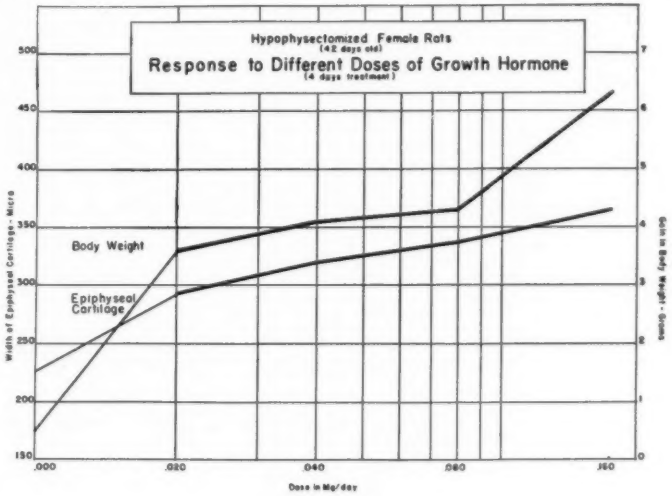

FIGURE 3

the factors are plotted on a semi-logarithmic scale. The means of the cartilage response fall in an approximately straight line while the means of the body weight response show greater variability.

D. Discussion

Ross and McLean (1940) suggest the use of qualitative changes occurring in the epiphyseal cartilage and the associated histological complex as a measure of growth hormone activity. While the application of criteria similar to those used by Ross and McLean show the essential character of the response evoked with each dose level,

such criteria leave too great a subjective element in an attempt to establish a quantitative relationship. In addition stimulation of osteogenesis is not marked after as short an injection period as four days (Figure 2). Therefore, one aspect of the total response, the increase in the thickness of the epiphyseal cartilage, was chosen as an index because it could be measured directly.

Freud, Levie, and Kroon's (1939) method of estimating growth hormone activity by measuring tail growth with skiagrams seems, at first glance, to incorporate the same advantage as the method described in this paper. Tail growth, however, does not immediately cease after hypophysectomy in the young rat. It may continue for as long as six weeks (Simpson, 1941), and this growth may vary considerably. While such variations can be eliminated by the use of adequate controls, the presence of such a factor limits the sensitivity if not the reliability of such an index. Changes in the thickness of the epiphyseal plates reflect disturbances in the equilibrium of chondrogenesis and osteogenesis. Such changes reveal positive and negative responses to growth hormone administration and hypophysectomy more directly and rapidly than measurements of tail length increase which depends on a summation of many factors.

The magnitude of the increase of epiphyseal cartilage width stimulated by the lowest dose used, 20 micrograms, seems to indicate that the minimal effective dose has not been reached. The demonstration of such a marked response of cartilage width in four days to 20 micrograms while the *MED* of this same growth hormone preparation in units based on body weight gain in 10 days was 43 micrograms serves as adequate evidence for the greater sensitivity of this index. While the body weight gain in the 4-day injection period (see Table 1) also shows a definite separation between the hypophysectomized controls and the group injected with 20 micrograms, this difference assumes little significance in view of the data on hundreds of hypophysectomized rats at this laboratory. The weight gains of all of the hypophysectomized animals, controls and injected, used in this experiment fall within the range of variations found in uninjected hypophysectomized rats of this age. The only group that may show a significant increase is the one receiving 160 micrograms. Conversely, histological data on over 50 hypophysectomized rats reveal a constant regression in epiphyseal cartilage width that bears little, if any, relation to the variable increase or loss in weight following hypophysectomy.

While observations on the size of the proximal cartilage plate of the tibia have been stressed in this paper, the complete picture of stimulation (Figure 2) revealed histologically makes necessary a further refutation of the term "chondrotrophic" hormone suggested as a substitute for growth hormone by Freud, Levie, and Kroon (1939). The same school of workers (Laqueur, Dingemanse, and Freud 1941) have recently clarified the limitations of the term "chondrotrophic."

E. Conclusions

1. After growth hormone administration at levels which do not significantly affect body weight in a four-day period, the proximal epiphyseal cartilage of the tibia of the young hypophysectomized rat, when examined histologically, revealed definite stimulation.

2. Daily injections of four dose levels of growth hormone to four groups of young hypophysectomized female rats from the 38th day to autopsy on the 42nd day resulted in greater widening of the epiphyseal cartilage with each increase in dose.

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Growth, 1941, **5**, 449-456.

THE EARLY EFFECT OF HYPOPHYSECTOMY AND OF IMMEDIATE GROWTH HORMONE THERAPY ON ENDOCHONDRAL BONE FORMATION

HERMANN BECKS, EDWIN A. KIBRICK, WALTER MARX, AND HERBERT M. EVANS

Divisions of Dental Medicine and Anatomy, the Institute of Experimental Biology, and the George Williams Hooper Foundation for Medical Research, University of California, San Francisco and Berkeley, California
(Received for publication, October 28, 1941)

A. INTRODUCTION

The effects of hypophysectomy on the epiphyseal cartilages of the young rat have been described by a number of workers (Freud, Levie, and Kroon, 1939; Ray, Evans, and Becks, 1941; Kibrick, Becks, Marx and Evans, 1941). These investigations have established the relation of the anterior pituitary and its growth hormone to the growth of the young rat. Freud and Dingemanse (1940) and Van Eck and Freud (1941) and Ray, Evans, and Becks (1941*a*) have summarized the essential characteristics of this relationship. In brief, hypophysectomy in the very young rat, under two weeks of age, slows but does not completely stop bone growth as indicated by the histology of the epiphyseal cartilage. During the "infantile period" (Freud and Dingemanse, 1940) of the first few weeks, growth is relatively independent of the pituitary secretions. After the first month, hypophysectomy results in a cessation of growth that is complete after a very short period. Thus the anterior pituitary assumes relatively complete control of skeletal growth as well as the growth of all the body tissues after the first three or four weeks in the life of the rat.

Heretofore, the effects of hypophysectomy on the histology of the long bones have been observed after a relatively long postoperative period. Ray, Evans, and Becks (1941*a*) have described the resorption of bony trabeculae that is found three weeks after hypophysectomy. Kibrick, Becks, Marx, and Evans (1941) have confirmed this finding. Before this phenomenon had been noted, the control of skeletal growth by growth hormone had seemed relatively simple. If growth hormone were present, growth continued, if it were removed, growth ceased.

Resorption of bone after the removal of the hypophysis, however, suggests the presence of an additional factor. Since the observations described above were made after a static histological condition had been reached, no clue to the actual mode of resorption was noted.

The converse problem, the effects of growth hormone administration on the skeletal growth of hypophysectomized rats, has also been investigated (Freud, Levie, and Kroon, 1939; Ray, Evans, and Becks, 1941; Kibrick, Becks, Marx, and Evans, 1941). In most cases a post-operative interval of one to two weeks preceded the administration of growth hormone. Therefore these investigations were primarily concerned with the repair of the skeletal changes that followed hypophysectomy.

In an effort to uncover the initial effects of hypophysectomy and investigate the problem of preventing such changes by immediate replacement therapy with growth hormone, the present study was undertaken.

B. Materials and Methods

Eighteen immature female rats were divided into three equal groups of six. Two groups were hypophysectomized on the 26th day. One of these hypophysectomized groups received four daily intraperitoneal injections of 40 micrograms of a cysteine-treated globulin growth hormone fraction beginning on the day of operation while the other remained untreated. All animals were autopsied on the 30th day.

The growth hormone preparation used was the same as that used by Kibrick, Becks, Marx, and Evans (1941) in their investigation of the effects of different dose levels of growth hormone on the epiphyseal cartilage of the proximal tibia of hypophysectomized rats. The pre-operative and post-operative diet was also the same.

Central sagittal sections of the proximal end of the right tibia of all animals were examined histologically after acid alcohol decalcification, nitrocellulose imbedding, and hematoxylin and eosin staining (Kibrick, 1941). A calibrated eyepiece micrometer was used for measuring the epiphyseal cartilage width.

C. Results

Four days after hypophysectomy a shrinkage in the width of the epiphyseal cartilage of the proximal tibia is apparent (compare Figure 1, Sections 1 and 2, and Table 1). The hyaline cartilage at the epiphyseal end of the cartilage disc—the so-called resting zone—is

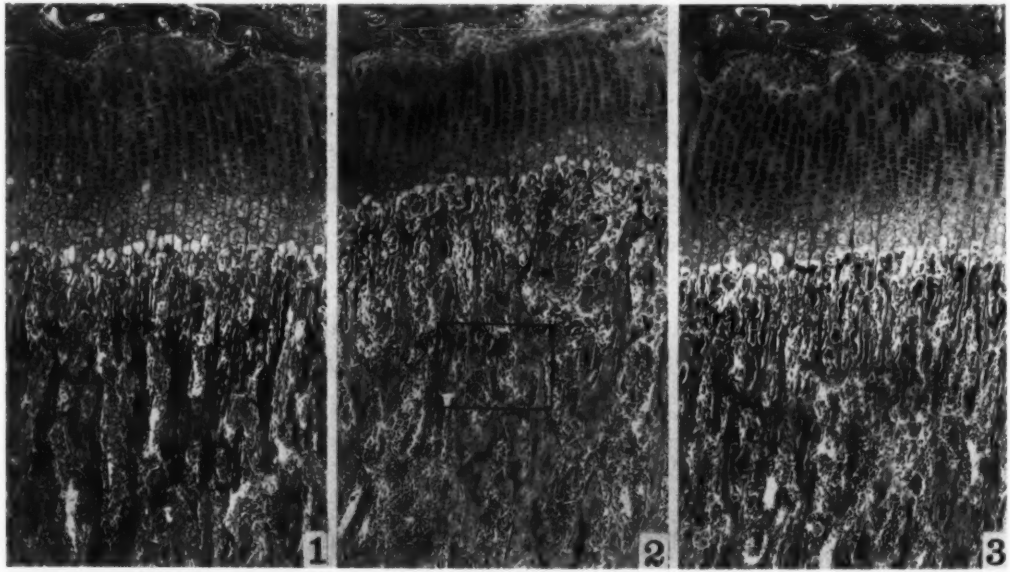

FIGURE 1

(Hematoxylin-eosin stain. All sections cut at 10 micra)

1. Central sagittal section of the proximal epiphyseal cartilage from the tibia of a normal 30-day-old female rat. X100
2. Central sagittal section of the proximal epiphyseal cartilage from the tibia of a female rat hypophysectomized on the 26th day and autopsied on the 30th day. X100
3. Central sagittal section of the proximal epiphyseal cartilage from the tibia of a female rat, hypophysectomized on the 26th day, injected daily with growth hormone from the 26th day to autopsy on the 30th day. X100

TABLE 1

Group	Animal number	Initial weight	Wt. gain from day of oper. (4 days), gm.	Width of epiphyseal cartilage in μ	Difference between Means
Normal controls	B6073	57	17	452	
	G6068	61	18	530	
	W6011	55	19	468	
	GH6093	62	19	484	
	BH6028	63	17	499	
	G6104	54	17	468	
			$m = 17.8$	$m = 484 \pm 11.4$	
					141 ± 16.1 (8.76)*
Hypophysectomized controls	BH6025	65	9	359	
	W5955	55	5	343	
	W6059	57	7	312	
	GH6062	58	8	328	
	G6080	55	7	390	
	W6087	64	5	328	
			$m = 6.8$	$m = 343 \pm 11.4$	
					131 ± 13.7 (9.49)*
Hypophysectomized +growth hormone 40 *σ/IP/* daily/4 days	GH6061	55	7	452	
	B6072	60	9	468	
	BH5985	55	11	468	
	W6086	60	9	499	
	GH6094	65	7	452	
	W6098	58	14	499	
			$m = 9.5$	$m = 473 \pm 8.7$	

*Ratio of significance.

unaffected. The columns of proliferating basophilic, cartilage cells are hypoplastic and the cells are more closely approximated than those in the normal control. The transition of proliferating cells to vesiculated cells in each column is more abrupt in the hypophysectomized animal. In addition, the number and size of vesiculated cells is decreased.

Below the epiphyseal disc, the disappearance of the normal pattern of long vertical bony trabeculae and the arrangement of the blood vessels and osteoblasts indicate the beginning of a disruptive process. The osteoblasts appear little affected but careful study under higher magnification reveals an unusual number of osteoclasts imbedded in the new bone of almost all of the trabeculae of the primary and

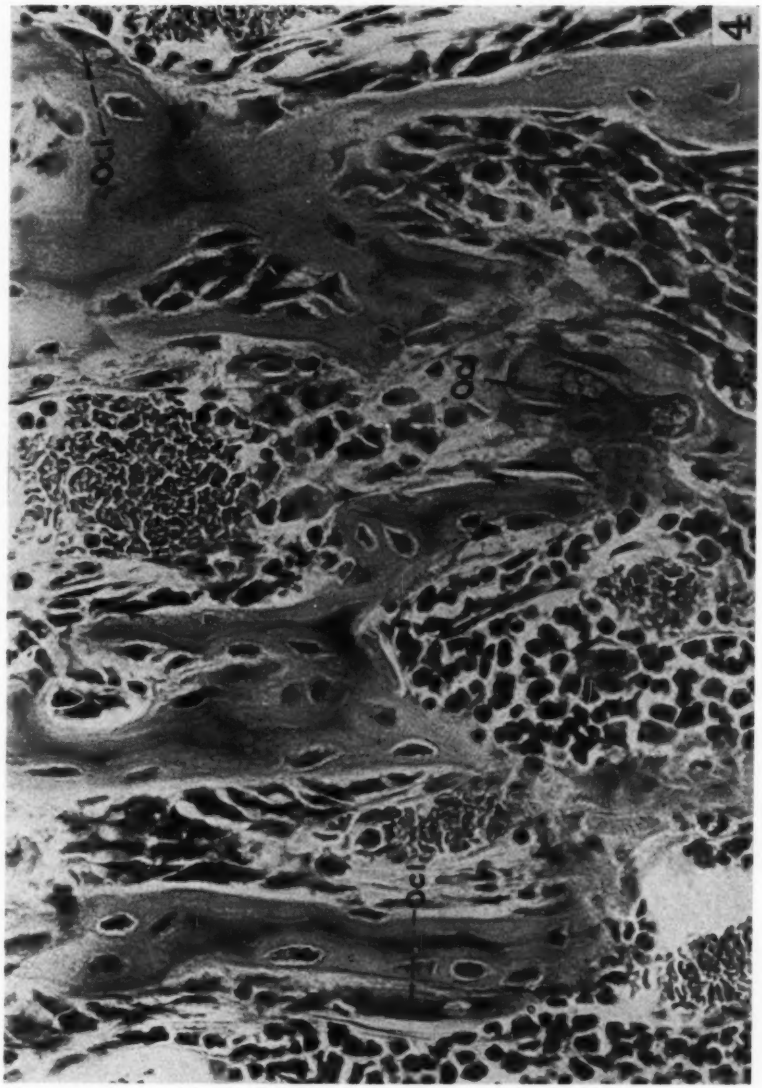

FIGURE 2

4. High power view of field outlined in Figure 1, Section 2. [Note the extensive vacuolization of the osteoclasts (Ocl.) X485.]

secondary spongiosa (Figure 2, Section 4). The number of osteoclasts visible in each high power field of the hypophysectomized animal greatly exceeds the number visible in similar fields of the normal control. In addition, the cytology of the osteoclast found in the hypophysectomized animal suggests great activity. The numerous nuclei are vesicular and stained lightly with hematoxylin. Many clear vacuoles are scattered throughout the delicate, filmy cytoplasm. In many cases, the eosinophilia of the cytoplasm closely approximates that of the new bone against which it lies so that it is often difficult to separate the two clearly.

In summation, regressive changes in the epiphyseal cartilage and its associated histological complex are visible four days after hypophysectomy. This is shown by a hypoplasia of the cartilage disc and the appearance of many osteoclasts in the primary and secondary spongiosa.

When the hypophysectomized animal treated with growth hormone is compared with the normal control, no significant histological difference can be seen (see Figure 1, Sections 1 and 3). Measurements of cartilage width confirm the impressions derived from the study of the histology of the two groups (Table 1).

It is interesting to note that growth hormone administered during the four days immediately following hypophysectomy maintained the normal histological appearance of the proximal tibia without maintaining body weight gains at the level of the normal control.

D. Discussion

In all three age groups, 55, 88, and 150 days, Ray, Evans, and Becks (1941*a*) noted that hypophysectomy produced a reduction in width of the epiphyseal cartilage. This reduction in width was described as the result of an active process rather than a passive cessation of growth. They also noted changes in the number and size of bony trabeculae beneath the epiphyseal disc that indicated resorption of bone.

The observations reported in this paper supply the missing gaps in the picture of changes in bone growth following hypophysectomy. An active catabolic process appears in the young growing bone shortly after the removal of the hypophysis. Anabolic activity is still great. Cuboidal and columnar osteoblasts are present in large numbers. But a shift in the equilibrium of bone deposition and resorption has oc-

curred. Many newly formed osteoclasts appear. Frequently the structure of these polynuclear giant cells lends weight to the view that they arise from a number of fused osteoblasts. Whether they arise from fused osteoblasts or appear as new histological elements derived from embryonic connective tissue is not important here. The increase in the ratio of osteoclasts to osteoblasts is indicative of the trend toward bone resorption rather than bone deposition.

The reduction in width of the epiphyseal cartilage seems to be the result of two factors. The crowding together of the cartilage cells indicates a decreased formation of matrix. The appearance of pyknotic nuclei in the proliferating cartilage cells suggests the beginning of atrophy. In addition, the extent of the capillary invasion and erosion of the vesiculated cartilage cells has increased. Thus, a passive cessation of growth and an active phase of destruction of the epiphyseal cartilage has appeared as a result of hypophysectomy.

All of the regressive changes in the epiphyseal cartilage and its associated bony trabeculae were prevented by the daily administration of 40 micrograms of a purified growth hormone preparation from the day of operation to the day of autopsy. The loss of weight increment following hypophysectomy was not completely prevented by the dose of growth hormone administered. Although it is probable that the operative procedure resulted in a temporary reduction of food consumption, the fact that the skeletal structures were maintained despite any such effect is highly significant. It may be that this response of the skeletal system indicates a specific affinity or sensitivity to the growth hormone. Kibrick, Becks, Marx, and Evans (1941) demonstrated this greater sensitivity of the epiphyseal cartilage to growth hormone by their work on the reparative action of growth hormone after regression due to hypophysectomy. The maintenance of the skeletal structure observed in the present investigation, however, establishes such a relationship more firmly.

E. Conclusions

1. Four days after the removal of the pituitary body, histological examination of the proximal end of the young rat tibia revealed a hypoplasia of the epiphyseal cartilage and an increased number of osteoclasts imbedded in the bony trabeculae beneath the epiphyseal cartilage. Thus an active phase of bone resorption was initiated in addition to the retardation of endochondral bone formation that follows hypophysectomy.

2. Daily administration of purified growth hormone (cysteine treated globulin fraction) from the day of hypophysectomy at 26 days to autopsy four days later prevented the skeletal regression that follows hypophysectomy.

3. Skeletal structures were thus maintained after hypophysectomy by a dose level of growth hormone inadequate for maintaining normal body weight gains.

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Bergmann, M., *Rockefeller Institute*, 66th St. and York Ave., New York, N. Y.
Bernheim, Alice R., 1009 Park Ave., New York, N. Y.
Berrill, N. J., *McGill University*, Montreal, Quebec, Canada.
Blakeslee, A. F., *Carnegie Institution of Washington, Department of Genetics*, Cold Springs Harbor, L. I., N. Y.
Blandau, Richard J., *Arnold Biological Laboratory, Brown University*, Providence, R. I.
Bloom, William, *Department of Anatomy, University of Chicago*, Chicago, Ill.
Blount, Raymond F., *Department of Anatomy, University of Minnesota*, Minneapolis, Minn.
Boell, E. J., *Yale University, Osborn Zoölogical Laboratory*, New Haven, Conn.
Von Bonin, Gerhardt, *Dpt. of Anatomy, Univ. of Illinois*, Chicago, Ill.
Bonner, John T., *Harvard Biological Laboratories*, Cambridge, Mass.
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Briggs, Janet B., *c/o Zoölogy Department, McGill University*, Montreal, P. Q., Canada.
Briggs, Robert W., *Department of Zoölogy, McGill University*, Montreal, Canada.
Brill, Edmund R., *Harvard Biological Laboratories*, Cambridge, Mass.
Brody, Samuel, *Dairy Building, University of Missouri*, Columbia, Mo.
Brown, Alice L., *"The Ontario,"* 2853, Ontario Road, Washington, D. C.
Brown, Frank A., Jr., *Department of Zoölogy, Northwestern University*, Evanston, Ill.
Buchanan, J. W., *Department of Zoölogy, Northwestern University*, Evanston, Ill.
Burkette, Josephine L., 2301 N. Kedvale Ave., Chicago, Ill.
Burr, H. S., *Yale University*, New Haven, Conn.

Butler, Elizabeth, *Vassar College*, Poughkeepsie, N. Y.
Butler, E. G., *Department of Biology, Princeton University*, Princeton, N. J.
Caldwell, Otis W., *Boyce Thompson Institute*, Yonkers, N. Y.
Carpenter, Esther, *Department of Zoölogy, Smith College*, Northampton, Mass.
Castle, Edward S., *Biological Laboratories, Harvard University*, Cambridge, Mass.
Chapman, S. Stephen, *Lankenau Hospital Res. Inst.*, Corinthian and Girard Ave., Philadelphia, Pa.
Chase, Barbara L., *Hoyt Hall, c/o Dr. E. P. Little*, Exeter, N. H.
Chase, Herman B., *Department of Zoölogy, University of Illinois*, Urbana, Ill.
Child, C. M., *Jordan Hall, Stanford University*, Calif.
Child, George P., *Amherst College*, Amherst, Mass.
Clausen, Harry J., *University of Colorado, School of Medicine and Hospitals*, 4200 East Ninth Ave., Denver, Colorado.
Coe, Wesley R., *Department of Zoölogy, Yale University*, New Haven, Conn.
Cohen, A. L., *Hopkins Marine Station*, Pacific Grove, Calif.
Colwin, Arthur L., *Queens College*, Flushing, N. Y.
Copeland, D. Eugene, *Department of Zoölogy, University of North Carolina*, Chapel Hill, N. Carolina.
Copenhaver, W. M., *College Physicians and Surgeons*, 630 West 168th St., New York, N. Y.
Costello, Donald P., *University of North Carolina*, Chapel Hill, N. Carolina.
Courtis, S. A., 9110 Dwight Ave., Detroit, Mich.
Danforth, C. H., *Department of Anatomy, Stanford University*, Calif.
Daniel, J. Frank, *Department of Zoölogy, University of California*, Berkeley, Calif.
Davenport, Charles B., Cold Spring Harbor, L. I., N. Y.
Dawson, Alden B., *Biological Laboratories, Harvard University*, Cambridge, Mass.
Dawson, Ray F., *University of Missouri*, Columbia, Mo.
Deuber, C. G., *Osborn Botanical Laboratory, Yale University*, New Haven, Conn.
Denues, A. R. T., *c/o U. S. Bureau of Mines*, Bruceton, Pa.
Dove, W. Franklin, *Biological Laboratory, Experiment Station, University of Maine*, Orono, Maine.
Duggar, B. M., *Department of Botany, University of Wisconsin*, Madison, Wis.
Dunning, W. F., *Wayne University College of Medicine*, Detroit, Mich.
Drury, H. F., 8 Chapman Place, Cambridge, Mass.
Dunn, L. C., *Columbia University*, New York, N. Y.
Eakin, Richard M., *Department of Zoölogy, University of California*, Berkeley, Calif.
Eastlick, Herbert L., *State College of Washington*, Pullman, Wash.
Emerson, Henry S., *Department of Anatomy, University of Michigan*, Ann Arbor, Mich.
Etkin, William, 1565 Waring Ave., New York, N. Y.
Evans, Hiram J., *Biological Laboratories*, Cambridge, Mass.
Farr, Wanda K., *American Cyanamid Co.*, Stamford, Conn.
Foust, H. L., *Iowa State College*, Ames, Iowa.
Frank, L. K., 565 Park Ave., New York, N. Y.

Fremont-Smith, Frank, *Josiah Macy, Jr., Foundation*, 565 Park Ave., New York, N. Y.
Friedman, Robert S., 60 Hyslop Rd., Brookline, Mass.
Gardiner, Mary S., *Bryn Mawr College*, Bryn Mawr, Pa.
Gast, P. R., *Harvard Forest*, Petersham, Mass.
Gillette, Roy, *Washington University*, St. Louis, Mo.
Glaser, Otto C., *Amherst College*, Amherst, Mass.
Goerner, Alfred, 366 Sterling Place, Brooklyn, N. Y.
Goldforb, A. J., *College of the City of New York*, New York, N. Y.
Goodrich, Hubert B., Biological Laboratory, *Wesleyan University*, Middletown, Conn.
Gortner, Ross A., *University of Minnesota, University Farm*, St. Paul, Minn.
Grant, Ronald, *McGill University*, Montreal, Canada.
Greene, Harry S. N., *Yale University, School of Medicine*, New Haven, Conn.
Gregory, Paul W., *University of California*, Davis, Calif.
Guthrie, Mary J., *University of Missouri*, Columbia, Mo.
Guyer, M. F., *Department of Zoölogy, University of Wisconsin*, Madison, Wis.
Haagen-Smit, A. J., *California Institute of Technology*, Pasadena, Calif.
Hall, Ada, *Wellesley College*, Wellesley, Mass.
Hamburger, Viktor, *Department of Zoölogy, Washington University*, St. Louis, Mo.
Hamilton, Howard L., *Department of Biology, The Johns Hopkins University*, Baltimore, Maryland.
Hammett, F. S., *Fiddlers Point*, Sarasota, Florida, and Provincetown, Mass.
Hamner, Karl C., *U. S. Department of Agriculture, Bureau of Plant Industry*, Ithaca, N. Y.
Handford, S. Wing, *New York University College of Dentistry*, New York, N. Y.
Hansen, Ira B., *Zoölogy Department, The George Washington University*, Washington, D. C.
Hard, W. L., *Department of Zoölogy, University of Maryland*, College Park, Md.
Harrison, Ross G., *Osborn Zoölogical Laboratory, Yale University*, New Haven, Conn.
Hartung, Ernest, 77 Perkins, *Harvard University, Cambridge*, Mass.
Harvey, Ethel B., 48 Cleveland Lane, Princeton, N. J.
Harvey, E. Newton, *Guyot Hall*, Princeton, N. J.
Harvey, Samuel C., 789 Howard Ave., New Haven, Conn.
Henshaw, P. S., *National Cancer Institute*, Bethesda, Md.
Hersh, A. H., *Department of Biology, Western Reserve University*, Cleveland, Ohio.
Hess, Walter, N., *Hamilton College*, Clinton, N. Y.
Hiesey, William M., *Carnegie Laboratory, Stanford University*, Calif.
Hoadley, Leigh, *Biological Laboratories, Harvard University*, Cambridge, Mass.
Hockenyos, George L., *Sentinel Insect Control Laboratory*, 213 E. Jefferson St., Springfield, Ill.
Hoobler, I. M., *Children's Fund of Michigan*, 660 Frederick St., Detroit, Mich.
Hooker, Davenport, *University of Pittsburgh*, Pittsburgh, Pa.
Huettner, Alfred H., *Department of Biology, Queens College*, Flushing, N. Y.
Humphrey, Rufus R., 24 High St., Buffalo, N. Y.

Hunt, Thomas E., *Department of Anatomy, University of Alabama,* University, Ala.
Huskins, C. Leonard, *Department of Genetics, McGill University,* Montreal, Que., Canada.
Hutt, Frederick B., *Department of Zoölogy and of Poultry Husbandry, Cornell University,* Ithaca, N. Y.
Irwin, M. R., *Department of Genetics, University of Wisconsin,* Madison, Wis.
Jaap, R. George, 129 Kings Highway, Stillwater, Okla.
Jaffe, H. L., 1919 Madison Ave., New York, N. Y.
Kaan, Helen W., *Department of Zoölogy, Wellesley College,* Wellesley, Mass.
Kaiser, Samuel, *Brooklyn College,* Brooklyn, N. Y.
Kaplan, H. M., 30 Irving St., Cambridge, Mass.
Kempton, Rudolf T., *Department of Zoölogy, Vassar College,* Poughkeepsie, N. Y.
Kidder, George W., *Arnold Biological Laboratory, Brown University,* Providence, R. I.
Kille, Frank R., *Swarthmore College, Martin Biological Laboratory,* Swarthmore, Pa.
Krems, Abraham D., 4536 Forest Park Blvd., St. Louis, Mo.
Landauer, Walter, *Atwater Laboratory, University of Connecticut,* Storrs, Conn.
Latimer, Homer B., *Department of Anatomy, University of Kansas,* Lawrence, Kans.
Lerner, Michael, *University of California,* Berkeley, Calif.
Lewis, Warren H., *The Wistar Institute,* Philadelphia, Pa.
Lillie, Frank R., 5801 Kenwood Ave., Chicago, Ill.
Lillie, Ralph S., 5545 Kenwood Ave., Chicago, Ill.
Link, George, K. K., *Department of Botany, University of Chicago,* Chicago, Ill.
Lisco, H., *Department of Pathology, Harvard Medical School,* Boston, Mass.
Little, Clarence C., *Roscoe B. Jackson Memorial Laboratory,* Box 558, Bar Harbor, Maine.
Loeb, Leo, *Washington University Medical School,* St. Louis, Mo.
Loomis, W. E., *Department of Botany, Iowa State College,* Ames, Iowa.
Ludlum, S. DeW., Gladwyne, Pa.
Lurie, Max B., *University of Pennsylvania, Henry Phipps Institute,* Seventh and Lombard Sts., Philadelphia, Pa.
McEwen, R. S., *Oberlin College,* Oberlin, Ohio.
McGraw, Myrtle, *Normal Child Development Study, Babies Hospital,* 167th St. and Broadway, New York, N. Y.
McLean, Franklin C., *Department of Physiology, University of Chicago,* Chicago Ill.
MacDowell, E. C., *Carnegie Institution of Washington,* Cold Spring Harbor, N. Y
Marvin, James W., 91 South Willard St., Burlington, Vt.
Mast, S. O., *Department of Zoölogy, Johns Hopkins University,* Baltimore, Md.
Mead, Albert D., 283 Wayland Ave., Providence, R. I.
Miller, Bernard J., *Lankenau Hospital Research Institute,* Philadelphia, Pa.
Miller, James, *Department of Anatomy, University of Michigan, Medical School,* Ann Arbor, Mich.
Murphy, James B., *Rockefeller Institute,* 66th St. and York Ave., New York, N. Y.

Murray, Margaret R., 630 W. 168 St., New York, N. Y.
Nabrit, S. Milton, *Atlanta University*, Atlanta, Ga.
Needham, Joseph, *Cambridge University*, England.
Nathanson, Y., 1737 Chestnut St., Philadelphia, Pa.
Northrop, F. S. C., *Yale University*, New Haven, Conn.
Okkelberg, Peter, *University of Michigan*, Ann Arbor, Mich.
Olson, Rodney A., *Chesapeake Biological Laboratory*, Solomons, Md.
Oppenheimer, Jane M., *Department of Biology, Bryn Mawr College*, Bryn Mawr, Pa.
Padis, Nicholas, *Lankenau Hospital*, Philadelphia, Pa.
Palmer, Carroll E., *U. S. Public Health Service, National Institute of Health*, Bethesda, Md.
Parker, G. H., *Harvard University*, Cambridge, Mass.
Parmenter, Charles L., *University of Pennsylvania, Zöology Building*, Philadelphia, Pa.
Peebles, Florence, *Chapman College*, Los Angeles, Calif.
Pincus, Gregory, *Department of Physiology, Clark University*, 950 Main St., Worcester, Mass.
Pollister, A. W., *Department of Zoölogy, Columbia University*, New York, N. Y.
Porter, Keith R., *Rockefeller Institute*, 66th and York Ave., New York, N. Y.
Potter, James S., *Carnegie Institution, Department of Genetics*, Cold Spring Harbor, L. I., N. Y.
Rabson, S. Milton, 166 W. 72nd St., New York, N. Y.
Rahn, Otto, *Dairy Building, Cornell University*, Ithaca, N. Y.
Raper, Kenneth B., *Northern Regional Laboratory, U. S. Department of Agriculture*, Peoria, Ill.
Reed, C. I., *Department of Physiology, University of Illinois, College of Medicine*, 1853 W. Polk St., Chicago, Ill.
Reed, Howard S., *Division of Plant Nutrition, University of California*, Berkeley, Calif.
Reed, Sheldon C., *Biological Laboratories, Harvard University*, Cambridge, Mass.
Reimann, Stanley P., *The Lankenau Hospital, Research Institute*, S. W. Cor. Corinthian and Girard Aves., Philadelphia, Pa.
Richards, O. W., 19 Doat St., Buffalo, N. Y.
Richardson, Dorothy, *Rockford College*, Rockford, Ill.
Riddle, Oscar, *Carnegie Institution*, Cold Spring Harbor, N. Y.
Riker, A. J., *Department of Plant Pathology, University of Wisconsin*, Madison Wis.
Risley, Paul L., *Zoölogy Department, State University of Iowa*, Iowa City, Iowa.
Roberts, R. H., *University of Wisconsin*, Madison, Wis.
Royal, Lester, Rossford, Ohio.
Rudnick, Dorothea, *Albertus Magnus College*, New Haven, Conn.
Rulon, Olin, *Department of Biology, Wayne University*, Detroit, Michigan.
Sacco, Paul, *Department of Botany, Pennsylvania State College*, State College, Pa.
Sander, Gerhard, *McGill University*, Montreal, Quebec, Canada.

Sandstrom, Carl J., *Department of Biology, New York University*, New York, N. Y.
Sawin, Paul B., *Biology Laboratory, Brown University*, Providence, R. I.
Sayles, Leonard P., *Department of Biology*, 139th St., and Convent Ave., New York, N. Y.
Scarth, G. W., *Department of Botany, McGill University*, Montreal, Canada
Schmitt, Francis O., *Massachusetts Institute of Technology*, Cambridge, Mass.
Schneider, Charles L., *Stanford University, Medical School*, Calif.
Schotte, Oscar E., *Department of Biology, Amherst College*, Amherst, Mass.
Scrimshaw, Nevin S., *Biological Laboratories, Harvard University*, Cambridge, Mass.
Shaw, Frank R., Fernald Hall, Amherst, Mass.
Shumway, Waldo, *Department of Zoölogy, University of Illinois*, Urbana, Ill.
Sideris, C. P., *Pineapple Research Institute of Hawaii*, Honolulu.
Sinden, Delphine Bowling, *Vassar College*, Poughkeepsie, N. Y.
Sinnott, Edmund W., *Osborn Botanical Laboratory, Yale University*, New Haven, Conn.
Skoog, Folke K., *Department of Biology, Johns Hopkins University*, Baltimore, Md.
Speese, Bernice M., 805 Carter Rd. Ral. Ct., Roanoke, Va.
Sponsler, O. L., *University of California in Los Angeles.*
Spratt, Nelson T., Jr., *Department of Biology, The Johns Hopkins University*, Baltimore, Md.
Stein, Kathryn F., *Mount Holyoke College*, South Hadley, Mass.
Steinhaus, Arthur H., 5315 Drexel Ave., Chicago, Ill.
Stern, Curt, *University of Rochester*, Rochester, N. Y.
Stewart, Harold L., *National Cancer Institute*, Bethesda, Md.
Swett, F. H., *Duke Hospital*, Durham, N. C.
Tartar, Vance, *Department of Zoölogy, University of Vermont*, Burlington, Vt.
Te Winkel, Lois E., *Department of Zoölogy, Smith College*, Northampton, Mass.
Thimann, Kenneth V., *Harvard Biological Laboratories*, Cambridge, Mass.
Toennies, Gerrit, *Lankenau Hospital*, Girard and Corinthian Ave., Philadelphia, Pa.
Trager, William, *Rockefeller Institute*, Princeton, N. J.
Trombetta, Vivian B., *Department of Botany, Smith College*, Northampton, Mass.
Tschudy, Robert H., *Scripps Institute of Oceanography*, La Jolla, Calif.
Tukey, Harold B., *N. Y. State Agricultural Experimental Station*, Geneva, N Y.
Twitty, V. C., *Stanford University*, Calif.
Uhlenhuth, Edward, *Department of Anatomy, University of Maryland Medical School*, Baltimore, Md.
Waddington, C. H., *Cambridge University, England.*
Warmke, H. E., *Department of Genetics, Carnegie Institution*, Cold Spring Harbor, N. Y.
Warren, A. E., *McMaster University*, Hamilton, Ontario.
Wassermann, Friedrich, *Department of Anatomy, University of Chicago*, Chicago, Ill.
Waterman, Allyn J., *Williams College*, Williamstown, Mass.
Weiss, Paul A., *University of Chicago*, Chicago, Ill.
Went, F. W., *California Institute of Technology*, Pasadena, Calif.

Wetmore, Ralph H., *Biological Laboratories, Harvard University,* Cambridge, Mass.
Whaley, W. G., *Barnard College,* New York, N. Y.
Whitaker, Douglas, M., *Department of Biology, Stanford University,* Calif.
White, Philip R., *The Rockefeller Institute,* Princeton, N. J.
Willier, Benjamin H., *Department of Biology, Johns Hopkins University,* Baltimore, Md.
Wilson, J. Walter, 282 Doyle Ave., Providence, R. I.
Wilson, Louise Palmer, *Wellesley College,* Wellesley, Mass.
Woodger, J. H., *Middlesex Hospital Medical School, University of London,* London, England.
Woods, Philip W., *State Bureau of Health,* Augusta, Maine.
Woodside, Gilbert L., *Fernald Hall, Massachusetts State College,* Amherst, Mass.
Woolley, George, *Roscoe B. Jackson Memorial Laboratory,* Bar Harbor, Maine.
Wright, Sewall, *Department of Zoölogy, University of Chicago,* Chicago, Ill.
Yocum, L. Edwin, *Department of Botany, The George Washington University,* Washington, D. C.